Rev. Inst. Med. trop. São Paulo
37(4): 349-352, julho/agosto, 1995.

STUDIES ON HUMAN ANTI-RABIES IMMUNIZATION IN BRAZIL. I - EVALUATION OF THE 3 + 1 PRE-EXPOSURE VACCINATION SCHEDULE UNDER FIELD CONDITIONS.

Carlos Roberto ZANETTI, Luciana Botelho CHAVES, Andréa de Cássia Rodrigues SILVA, Luiza Marny LEE & Octavio Augusto de Carvalho PEREIRA

SUMMARY

The currently used pre-exposure anti-rabies immunization schedule in Brazil is the one called 3+1, employing suckling mouse brain vaccine (3 doses on alternate days and the last one on day 30).

Although satisfactory results were obtained in well controlled experimental groups using this immunization schedule, in our routine practice, VNA levels lower than 0.5 IU/ml are frequently found.

We studied the pre-exposure 3+1 schedule under field conditions in different cities on the State of São Paulo, Brazil, under variable and sometimes adverse circumstances, such as the use of different batches of vaccine with different titers, delivered, stored and administered under local conditions. Fifty out of 256 serum samples (19.5%) showed VNA titers lower than 0.5 IU/ml, but they were not distributed homogeneously among the localities studied.

While in some cities the results were completely satisfactory, in others almost 40% did not attain the minimum VNA titer required.

The results presented here, considered separately, question our currently used procedures for human pre-exposure anti-rabies immunization. The reasons determining this situation are discussed.

KEYWORDS: Rabies; Pre-exposure vaccination; suckling mouse brain anti-rabies vaccine.

INTRODUCTION

Since 1966, the WHO Expert Committee on Rabies recommends that pre-exposure immunization should be offered to persons at high risk of exposure, such as laboratory staff working with rabies virus, veterinarians, animal handlers, wildlife officers and other individuals who are living in or traveling to areas where rabies is endemic[9].

Since the determinations of the virus neutralizing antibody (VNA) response has become routine practice, the WHO expert committee on rabies considers, since 1978, that strict harmonization of immunization schedules is of minimum significance, as long as a value of at least 0.5 International Units per ml (IU/ml) is attained to demonstrate seroconversion[12]. After pre-exposure immunization, people at continuous risk of exposure should have a serum sample tested periodically (every 6 or 12 months) and a booster administered when the titer falls below that level[13].

The currently used rabies vaccine in Brazil, recommended by local official health authorities, is the suckling mouse brain vaccine, developed by FUENZALIDA & PALACIOS[7], and the pre-exposure immunization

This work was supported, in part, by CNPq and Secretary of Health of the State of São Paulo.
INSTITUTO PASTEUR, São Paulo, Brasil.
Correspondence to: Carlos Roberto Zanetti, Instituto Pasteur, Av. Paulista 393, 01311-000 São Paulo, SP, Brazil. Fax 55.11.289-0831.

schedule is the one called 3+1 (three doses on alternate days and an additional one 30 days later)[10].

Satisfactory results were obtained in well controlled experimental groups using this immunization regimen or very similar variants[3,4]. In our routine, however, VNA levels lower than 0.5 IU/ml are frequently found.

Since the purpose of this study was to evaluate the pre-exposure 3+1 schedule under field conditions, we studied the levels of neutralizing response of people submitted to this immunization schedule in different cities in the State of São Paulo, under variable and sometimes adverse circumstances such as the use of different batches of vaccine with different titers (although always with a titer of at least 0.6 IU/dose), delivered, stored and administered under local conditions.

MATERIALS AND METHODS

Subjects and vaccine

A total of 256 subjects (180 males and 76 females) whose professional activities justified pre-exposure treatment received the 3+1 schedule from May to December, 1993. They were vaccinated with the suckling mouse brain vaccine, prepared with CVS strain [potency value of at least 0.6 IU/dose as measured by the NIH test[11]], provided by the Instituto de Tecnologia do Paraná, TECPAR.

Serum samples and VNA determination

Serum samples were collected 20 to 30 days after the last rabies vaccine injection and submitted to the Instituto Pasteur, São Paulo, as a routine procedure for VNA determination.

Virus neutralizing antibodies against the PV strain were tested by the simplified fluorescence inhibition microtest as described elsewhere[5]. VNA is expressed as IU/ml using as reference an equine hyperimmune serum adjusted to a concentration of 200 IU/ml. In our routine, the reference serum included in each test was diluted in two series of twofold dilutions beginning with 1/2000 and 1/3000 and human sera were distributed among series of four wells of threefold dilutions beginning with 1/5. A pool of the 25 sera received from Itu was also included in a twofold dilution series.

RESULTS

Table 1 presents the number and percentages of the serum samples correlated with the detected VNA titers. Fifty out of the 256 serum samples (19.5%) showed VNA titers lower than 0.5 IU/ml whereas 185 (72.3%) presented results higher than 1.0 IU/ml.

TABLE 1
Correlation between the number and percentages of serum samples and the ranges of VNA titers.

	Range of VNA titers in International Units/ml			
	< 0.5	$\geq 0.5 \leq 1.0$	> 1.0	Total
Number of sera (%)	50 (19.5)	21 (8.2)	185 (72.3)	256

Table 2 shows the number of failures (titers below 0.5 IU/ml) among the total number of samples tested correlated with cities sending serum samples to Instituto Pasteur, São Paulo, for testing. As it can be observed, 229 serum samples came from 11 different cities that sent 5 or more samples each. Among them, Avaré, Espírito Santo do Pinhal, Iguape, Itu and Jaboticabal totaled 124 serum samples, with 113 (91.1%) results higher than 1.0 IU/ml and only two failures (1.6%). On the other hand, the failures in the sera from the other 6 cities were 40 of 105 samples (38.1%).

TABLE 2
Correlation between the number of failures (titers lower than 0.5 IU/ml) and the total number of samples tested.

Localities	Number of sera	
	VNA titers < 0.5 IU/ml	Total
1. Andradina	0	1
2. Apiaí	0	2
3. Araçatuba	6	35
4. Avaré	1	11
5. Botucatu	1	2
6. Bauru	0	1
7. Colina	5	5
8. Espirito Sto. Pinhal	1	49
9. Guarulhos	3	11
10. Iguape	0	17
11. Ilha Solteira	4	13
12. Itapeva	0	1
13. Itapira	1	1
14. Itú	0	25
15. Jaboticabal	0	22
16. Jardinópolis	0	1
17. Lorena	0	1
18. Marília	1	1
19. Mogi-Guaçu	1	1
20. Monte Alto	0	1
21. Pindamonhangaba	1	2
22. Pirassununga	11	15
23. Presidente Prudente	0	2
24. Ribeirão Preto	1	3
25. São João da Boa Vista	2	3
26. São José do Rio Preto	0	2
27. São Paulo	11	26
28. Rosana	0	1
29. Taiaçu	0	1

The best results were obtained in Itu with neutralizing titers of at least 1.68 IU/ml. The pool prepared with the 25 samples from Itu presented a VNA titer of 8.0 IU/ml.

DISCUSSION

Two aspects are particularly interesting in the present results:

1. The incidence of unsatisfactory results obtained with the pre-exposure schedule was too elevated in our routine work conditions.

Considering that this kind of immunization schedule is administered to subjects at continuous risk of exposure to rabies, who must therefore be maintained with high VNA titers in order to keep satisfactory levels of immunity, acceptance of failure numbers as high as 20% is extremely dangerous.

2. There was no homogeneous distribution of low VNA titer serum samples among the localities studied. While in some of them the results were completely satisfactory, in others, 40 in a total of 105 vaccines (38.1%) did not attain VNA titers of 0.5 IU/ml (Table 2).

Several reasons might have importantly influenced the results presented here. The vaccine routinely used in Brazil must contain at least 0.6 IU/dose, as measured by the NIH test[11]. Frequently, however, some batches present 2 or 3 IU/dose[1]. In addition, the NIH test is not very reproducible, showing wide variation in tests performed in duplicate[1,2].

Thus, it is difficult to establish the magnitude of the differences in real potency levels among the different vaccine batches delivered to the towns involved in this study during an eight-month period.

Adverse conditions of transport, storage or administration of the vaccine [injections into the gluteus region, still widely used, are now known to be less immunogenic than injections into the deltoid region[6,13]] may also have influenced the results. Some conditions may be even worse due to some local additional problems such as difficulty for patients to go to the doctor. In such cases, the vaccine doses are sometimes delivered to the patient, who must carry out the treatment on his own responsibility.

In addition, it has already been shown that significant differences in VNA titers can be obtained when serum samples from mice immunized with one vaccinal strain are submitted to serum neutralization against different ones, including those used in human vaccination such as PV and CVS[8].

Our results were obtained with the virus neutralization test against the PV strain. Nevertheless, the vaccine used in the state of São Paulo in 1993, when this study was performed, was produced with the CVS strain.

We obtained very good previous results employing only three doses of vaccine (days zero, 3 and 7). On that occasion, however, besides the fact that we worked with a highly controlled group, the vaccine used was produced with the PV strain and the neutralization tests were performed against the homologous strain[4].

The presently results, considered separately, question our currently used procedures for human pre-exposure anti-rabies immunization.

However, 98% seroconversion rates (with more than 90% of VNA titers higher than 1.0 IU/ml were obtained in 124 samples from various towns; in the 25 samples from Itu, the seroconversion rate was 100%, with individual VNA titers of at least 1.68 IU/ml and a mean of 8.0 IU/ml.

These results suggest that more than problems with the schedule, the vaccine or the serum neutralization test, local adverse conditions probably determined the elevated rate of failures, but the correct evaluation of the real participation of all the factors discussed depends on other studies, already underway.

However, caution is necessary when performing post-exposure treatment of previously vaccinated persons, since according to our official recommendations, subjects already submitted to the 3+1 schedule are regarded as previously immunized and should receive only a booster regimen.

RESUMO

Estudo sobre a imunização anti-rábica humana no Brazil. I - Avaliação do esquema de vacinação pré-exposição 3+1 em condições de campo.

O esquema de imunização anti-rábica pré-exposição mais comumente utilizado no Brasil é o chamado 3 + 1, com emprego de vacina produzida em tecido cerebral de camundongos recém-nascidos (3 doses em dias alternados e uma dose no dia 30).

Embora tenham sido obtidos bons resultados em grupos experimentais bem controlados com este esquema de vacinação, no Laboratório de Sorologia do Instituto Pasteur, São Paulo, são encontrados, com frequência, resultados inferiores a 0,5 UI/ml de anticorpos neutralizantes.

Estudamos o esquema pré-exposição 3+1 em condições de campo, em diferentes cidades do Estado de São Paulo, em condições variáveis e talvez adversas, tais como a utilização de diferentes lotes de vacinas, com diferentes títulos, entregues, estocadas e administradas nas condições locais. Cinquenta das 256 amostras de soro (19,5%) apresentaram títulos inferiores a 0,5 UI/ml, sem que houvesse, no entanto, uma distribuição homogênea entre as cidades estudadas.

Enquanto em algumas cidades os resultados foram amplamente satisfatórios, em outras quase 40% deles deixaram de atingir o nível mínimo desejável.

Os resultados aqui apresentados, se considerados isoladamente, tornam questionáveis nossos procedimentos atuais de vacinação anti-rábica humana pré-exposição. As razões determinantes desta situação são discutidas no trabalho.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mrs. Dolores Ayako Yoda for the technical assistence.

REFERENCES

1. ALBAS, A.; MOURÃO FUCHES, R.M.; FRAZATTI GALLINA, N.M. et al. - Termoestabilidade da vacina contra raiva, tipo Fuenzalida & Palacios, uso humano. **Rev. Inst. Med. trop. S. Paulo, 34:** 27-31, 1992.
2. BARTH, R.; GROSS-ALBENHAUSEN, E.; JAEGER, O. & MILCKE, L. - The antibody-binding-test, a useful method for quantitative determination of inactivated rabies virus antigen. **J.biol.Stand., 9:**81-89, 1981.
3. CHAMELET, E.L.B.; AZEVEDO, M.P.; FAVORETTO, S.R.; KERBRIE, S.V. & SOUZA, L.T.M. - Esquema reduzido de vacinação anti-rábica humana pré-exposição e avaliação de doses anuais de reforço. **Rev. Saúde publ.** (S. Paulo), **16:**144-148, 1982.
4. FAVORETTO, S.R.; CARRIERI, M.L.; TINO, M.S. et al. - Reduced schedule of human anti-rabies immunization with Fuenzalida & Palacios vaccine. Additional data. **Rev.Inst.Med. trop. S. Paulo, 35:** 281-284, 1993.
5. FAVORETTO, S.R.; CARRIERI, M.L.; TINO, M.S.; ZANETTI, C.R. & PEREIRA, O.A.C. - Simplified fluorescent inhibition microtest for the titration of rabies neutralizing antibodies. **Rev. Inst. Med. trop. S. Paulo, 35:**171-175, 1993.
6. FISHBEIN, D.B.; SAWYER, L.A.; REID-SANDEN, F.L. & WEIR, E.W. - Administration of human diploid-cell rabies vaccine in the gluteal area. **New Engl. J. Med., 318:**124-125, 1988.
7. FUENZALIDA, E. & PALACIOS, R. - Un método mejorado en la preparación de la vacuna antirábica. **Bol. Inst. bact. Chile, 8:**3-10, 1955.
8. LAFON, M.; BOURHYM, H. & SUREAU, P. - Immunity against the European bat rabies (Duvenhage) virus induced by rabies vaccines: an experimental study in mice. **Vaccine, 6:** 362-368, 1988.
9. ORGANISATION MONDIALE DE LA SANTÉ - Comité OMS d'experts de rage; cinquième rapport. Genéve, OMS, 1966. **Org. mond. Santé Ser. Rapp. techn.,** (321), 1966.
10. SÃO PAULO (Estado) Secretaria de Estado da Saúde - Norma Técnica SS 1/88. Tratamento preventivo anti-rábico humano. **Diário Oficial do Estado de São Paulo,** 11 Nov. 1988. Seção I, p.11.
11. SELIGMAN Jr., E.B. - Prueba de potencia NIH. **In:** KAPLAN, M.M. & KOPROWSKI, H. *La rabia: técnicas de laboratório.* 3ed. Ginebra, Organización Mundial de la Salud, 1976. p.294-302. (Org. mund. Salud Sér. Monogr., 23).
12. SINNECKER, H.; ATANASIU, P.; BAHMANYAR, M. et al. - Vaccine potency requirements for reduced immunization schedules and pre-exposure treatment. **Develop. biol. Stand., 40:**268-270, 1978
13. WORLD HEALTH ORGANIZATION - *WHO expert committee on rabies; eight report.* Geneva, WHO, 1992. **Wld. Hlth. Org. techn. Rep. Ser.,** (824), 1992.

Recebido para publicação em 09/01/1995
Aceito para publicação em 24/01/1995