

## Reduced Regimen for Human Pre-exposure Immunization against Rabies

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### ABSTRACT

The main aim of this study was to find a suitable rabies vaccination protocol which would allow usage of lower volumes of vaccine, fewer clinical visits and above all, enough serological effectiveness for high-risk individuals requiring pre-exposure immunization. Human diploid cell rabies vaccine was administered intradermally and intramuscularly in 60 previously unvaccinated volunteers, aged 19 to 21, following different vaccination protocols. The participants were divided into six groups, and rabies antibody titration was accomplished by ELISA. The comparison of the mean titers 30 days after termination of vaccination protocols shows that a protocol requiring only two clinical visits and ID injection of a total volume of only 0.3 ml of human diploid cell rabies vaccine (0.1 + 0.1 ml on day 0, one in each arm, and 0.1 ml on day 28) provides good serological response (6.92 IU/ml).

*Keywords:* Rabies, anti-rabies vaccine, human immunization

### INTRODUCTION

The choice of an appropriate anti-rabies vaccine is dictated by its safety, its immunization potentials and economic factors. Vaccines prepared from mammalian nervous tissues and from avian embryos have not been satisfactory because of complications associated with the former and the weak response induced by the latter [1, 2].

Human diploid cell culture vaccine used in Iran, Germany and other countries since 1976 is clearly better [1, 3-5]. Although very immunogenic and associated with minimal complications, its cost is a limiting factor for third world countries, wherein the highest incidence of rabies is found. Rabies exposed individuals in the economically underdeveloped countries will willingly accept any form of available vaccination because the fortunes of the disease are certain to be more bleak than any vaccine associated complications, but individuals requiring pre-exposure vaccination are more demanding on the quality of vaccine being used. The importance of this sort of vaccination for individuals at high risk of exposure cannot be over emphasized. Such individuals may become infected inadvertently without knowing that they have been ex-

posed and therefore not seek post-exposure treatment. Precisely this situation arose in Iran in 1991, leading to the death of a 39 years old veterinary technician. The man had no history of animal bites, but had 40 days prior to development of symptoms inserted his bare hands into the mouth of a rabid cow [6]. Infection by rabies virus was confirmed by detection of rabies antigen in brain tissue with use of fluorescent labeled anti-nucleocapsid antibodies.

The objective of this study was to determine a pre-exposure vaccination protocol using a human diploid cell culture rabies vaccine which provides sufficient protection at minimal cost. The minimization of cost was to be achieved primarily by decreasing total volume of vaccine injected per individual during the vaccination protocol. The goal is third world oriented given the economic limitations of third world countries.

### MATERIALS AND METHODS

Human diploid cell rabies vaccine produced by Pasteur Merieux Institute (Lyon, France) was used in this study. The vaccine is prepared from the supernatants of human embryonic lung using fibroblast cultures (WI-38) infected with the *Pitman-Moore*

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strain of rabies virus. Viruses in the culture are inactivated by beta-propiolactone. For vaccine preparation, viruses in the culture supernatant are concentrated, lyophilized and kept at 4°C. The lot number of the vaccines used was 130-822 and its antigenic value was 3.36 IU/dose/ml [7]. The lyophilized vaccine was reconstituted with sterile distilled water immediately before use.

Variable parameters in the vaccination protocols were injection route, schedule of injection and volume of vaccine used. Participants were informed volunteers among healthy first year male students (19-21 years old) of the College of Veterinary Medicine of the University of Tehran. None had previous to this study been exposed to any form of anti-rabies vaccination.

The students were divided into six groups (A-F) of 12 (Table 1). In only one group did all participants cooperated until the end of the study and therefore the effective number of participants per group ranged from 7 to 12. The vaccination protocol for each group is described below:

**Group A:** Three 1 ml intramuscular injections on days 0, 7 and 28. This was considered the control group as its vaccination protocol is prescribed by WHO Expert Committee on Rabies [8].

**Group B:** Three 0.1 ml intradermal injections on days 0, 7 and 28.

**Group C:** Three 0.1 ml intramuscular injections on days 0, 28 and 56.

**Group D:** Three 0.1 ml intradermal injections on days 0, 28 and 56.

**Group E:** Intramuscular injections on days 0, and 28; on day 0, two 1 ml injections were given, one in each arm and on day 28 a single 1 ml injection was given.

**Group F:** Intradermal injections on days 0 and 28; on day 0, two 0.1 ml injections were given, one in each arm and on day 28, a single 0.1 ml injection was given.

The criterion used for evaluation of vaccination protocol was rabies neutralizing antibody titers produced in vaccinated individuals. For titer determination the ELISA was performed using glycoprotein antigen coated microplates produced by Diagnostics Pasteur France (plattelia rage). Blood samples were obtained on days zero of vaccination,

30 days after the termination of the vaccination protocol and on one intermediate date. Sera from the samples were immediately prepared and stored at -20°C until day of assay. Samples were titrated in duplicate on at least two different occasions.

## RESULTS

No adverse reaction was observed in participants, except 3 individuals who have experienced redness at the site of inoculation, lethargy, and slight fever. these symptoms lasted only 3-4 days.

The most significant result of the study is reflected in comparison of mean titer of individuals of group F compared to group A, 30 days after termination of the respective protocols. Though the mean titer of group F (6.92 IU/ml) is somewhat lower than of group A (9.81 IU/ml), the level is adequate and well above the minimum protection level recommended by WHO (0.5 IU/ml) [8].

The desirability of this protocol lies in the fact that compared to the WHO recommended protocol, it requires only one tenth the volume of vaccine. The need for only two visits for vaccination in protocol F versus to three visits in protocol A is of added practical advantage.

In addition to above finding, two further observations on the data should be noted. First, there is some indication in previous publications that the intradermal route is preferable to the intramuscular one [9]. In this regard, group C and D whose protocols differ only in route of vaccine administration, can be compared. The 30 day Post-Vaccination titer of the intradermal route (group D) was slightly higher than that of the intramuscular route (group C: 3.68 IU/ml vs. 2.11 IU/ml respectively, Table I). Also the comparison of the 30 days Post-Vaccination titers of groups D and B suggests that comparable titers are achieved in the 0, 7, 28 and the 0, 28, 56 days schedules of ID vaccination. The rather high mean titer on day 42 in protocol D (4.04 IU/ml) was observed in three independent assays, was unexpected. The results are provided in Table 1.

## DISCUSSION

A vaccination protocol of two 0.1 ml injections, one in each arm on day 0. and a 0.1 ml injection on day 28 (group F) resulted in production of very reasonable rabies neutralizing antibody titers. Because

**Table 1.** Rabies antibody titers under various vaccination protocols.

Group	Participants	Vaccine used (ml)	Vaccination schedule Day/Volume (ml) <sup>a</sup>	Route <sup>b</sup>	Mean titer during vaccination <sup>c</sup>	Mean titer 30 days after vaccination <sup>c</sup>
A	11	3.0	0/1, 7/1, 28/1	I.M.	2.68	9.81
B	12	0.3	0/0.1, 7/0.1, 28/0.1	I.D.	1.29	3.95
C	9	0.3	0/0.1, 28/0.1, 56/0.1	I.M.	2.00	2.11
D	11	0.3	0/0.1, 28/0.1, 56/0.1	I.D.	4.04	3.68
E	10	3.0	0/1+1, 28/1	I.M.	1.80	6.75
F	7	0.3	0/0.1+0.1, 28/0.1	I.D.	1.70	6.92

<sup>a</sup> Days of vaccine administration are given above line and volumes administrated below the line; where two volumes are indicated, one volume was injected into each arm. <sup>b</sup>I.M. (intramuscular), I.D. (intradermal). <sup>c</sup>Titers are given in IU/ml serum. For groups A, B, E and F, blood samples were taken on day 14 and for groups C and D on day 42. The data for the latter two groups were chosen so to be after administration of second dose of the vaccine.

of a ten fold reduction in vaccine usage, the concomitant decrease in costs which this vaccination protocol allows, and the fact that two clinical visits for vaccination are required, its usage should be considered for individuals with significant risk of exposure to rabies virus. This group of individuals consists of laboratory staff working with rabies virus, veterinarians, animal handlers and wildlife officers, and other individuals who are living in or traveling to areas where rabies is endemic [8]. It would be worthy to do follow up studies in vaccinated individuals to determine duration of response. Response to later booster doses should also be studied.

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