ADVERSE EFFECT VERSUS QUALITY CONTROL OF THE FUENZALIDA-PALACIOS ANTIRABIES VACCINE

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SUMMARY

We evaluated the components of the Fuenzalida-Palacios antirabies vaccine, which is till used in most developing countries in human immunization for treatment and prophylaxis. This vaccine is prepared from newborn mouse brains at 1% concentration. Even though the vaccine is considered to have a low myelin content, it is not fully free of myelin or of other undesirable components that might trigger adverse effects after vaccination. The most severe effect is a post-vaccination neuroparalytic accident associated with Guillain-Barré syndrome. In the present study we demonstrate how the vaccines produced and distributed by different laboratories show different component patterns with different degrees of impurity and with varying protein concentrations, indicating that production processes can vary from one laboratory to another. These differences, which could be resolved using a better quality control process, may affect and impair immunization, with consequent risks and adverse effects after vaccination. We used crossed immunoelectrophoresis to evaluate and demonstrate the possibility of quality control in vaccine production, reducing the risk factors possibly involved in these immunizing products.

KEYWORDS: Adverse effect; Antirabies vaccine; Fuenzalida-Palacios; HDVC; Albumin; Crossed immunoelectrophoresis.

INTRODUCTION

Vaccines are applied at Public Health Services in order to prevent disease and minimize health costs. Like any other pharmacological medication, vaccines also pose some risks and therefore quality control of the production process and of the product to be released is needed to prevent as much as possible any risks these immunizing substances may pose.

In the case of control of human and canine rabies in developing countries such as Brazil, the vaccine used is the Fuenzalida-Palacios type[7], which is prepared from newborn mouse brains and is employed both for prophylaxis and treatment.

Approximately 150 thousand rabies cases are treated in Brazil and 25 thousand in the State of São Paulo[8]. Although vaccines produced in cell culture are available on the market, presenting a lower risk of development of Guillain-Barré syndrome[2], in this syndrome the patients present demyelinization after immunization with vaccines prepared with nervous tissue. Post-vaccination paralytic accidents may be irreversible, leading to patient death or, depending on the severity of the condition, to reversal followed or not by sequelae. In any case, adverse effects of the vaccine are known to occur and are a constant source of concern for the producing laboratories.

In 1997, 22,840 treatments were performed in the State of São Paulo[8] with the vaccine of the Fuenzalida-Palacios type and seven cases involving adverse post-vaccination events were reported[6], however the incidence is estimated about 1:8,000 cases[17].

In view of this situation, the objective of the present study was to evaluate the quality control of the vaccines distributed by the Health Department. It was not our objective to evaluate the vaccines in terms of efficacy and potency in the protection against rabies, but rather in terms of the nonspecific protein components they contain which may affect their immunogenic competence and which are probably responsible for the triggering of adverse effects after immunization.

MATERIAL AND METHODS

Crossed immunoelectrophoresis: We used the technique described by WEEKE[19], later developed by LAURELL[11] and adapted by CLARKE & FREEMAN[5] for the determination of protein complexes in cerebrospinal fluid or of abnormal protein patterns in serum. The technique was then standardized by NOGUEIRA & MONTAÑO[13] for the identification of immunogenic fractions of the Fuenzalida-Palacios antirabies vaccine in a case study of post-vaccinal neuroparalytic accidents.

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Electrophoresis on 7.5% polyacrylamide gel: We used the technique of LAEMMLI[10], widely used for the electrophoretic separation of proteins in general. The protein content of the vaccine samples was determined by the micromethod of BRADFORD[4].

MATERIAL

1) Electrophoresis source

2) Electrophoresis cuvette

3) Glass slides (50 x 70 mm)

4) Buffer solutions

5) Medium type agarose (Sigma)

6) Acrylamide, bisacrylamide, TEMED (Sigma)

7) Hyperimmune antirabies serum distributed by CEME (Central de Medicamentos).

Vaccine samples: we used samples of vaccines distributed by CEME and collected from several Public Health Services and also vaccines provided by the producing laboratories. Each vaccine lot was identified by number as indicated below. The names of the producing laboratory were not revealed for ethical reasons. We used 3 lots of vaccines from Brazilian laboratories and 2 lots of vaccines from laboratories in other Latin American countries. We also used a vaccine produced in Brazil using cell culture technology but for veterinary use. Each lot is identified below:

lot 1 (Brazilian manufacturer) Laboratory A

lot 2 (Brazilian manufacturer) Laboratory B

lot 3 (Brazilian manufacturer) Laboratory C

lot 4 (Brazilian manufacturer) Laboratory D (cell culture technology)

lot 5 (foreign manufactures) Laboratory E

lot 6 (foreign manufacturer) Laboratory F (vaccine concentration = 5%)

lot 7 (same manufacturer) Laboratory F (vaccine concentration = 2%)

lot 8 (same manufacturer) Laboratory F (vaccine concentration = 1%)

9) External controls: a) sphingomyelin (Sigma), b) bovine albumin, fraction V (Sigma); c) mouse serum, d) molecular weight standard (Pharmacia).

Procedure: The procedure used was that of NOGUEIRA[14].

RESULTS

Figure 1 shows the crossed immunoelectrophoretic patterns of the vaccine samples used in the present study. Figures 1A, B and C correspond to the vaccine lots produced in Brazil, Figure 1D shows the vaccine produced by cell culture and purchased on the market, and Figures 1E and F (5%, 2%, 1%) correspond to vaccines produced in other Latin American countries.
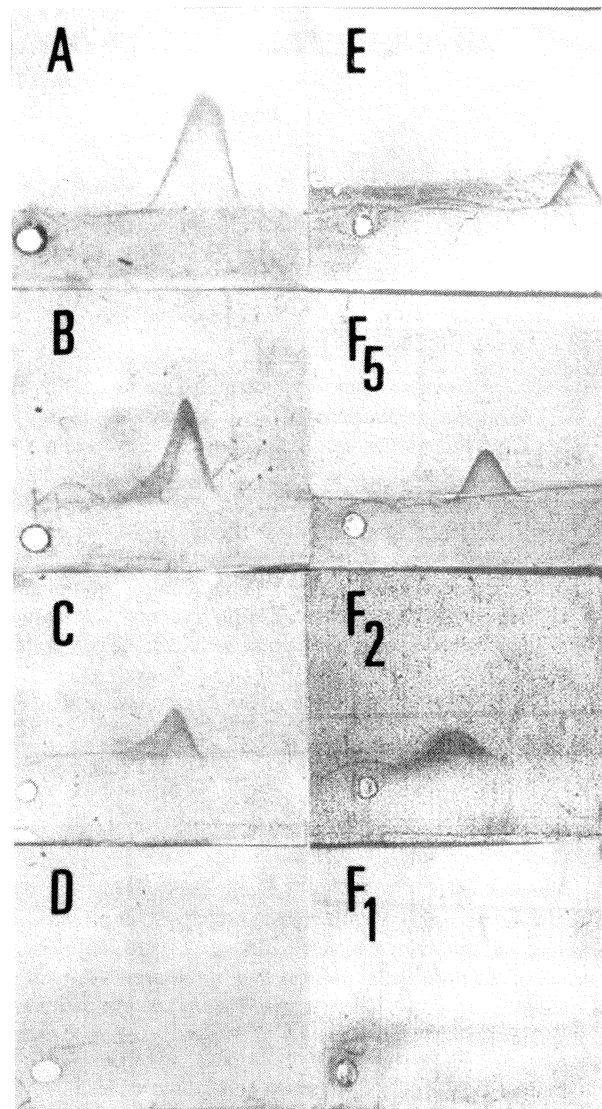

Fig. 1 - Crossed immunoelectrophoresis with hyperimmune antirabies serum incorporated into agarose gel (1:10, v/v) reacting with 5 µl of each vaccine dose: 1A) lot 1; 1B) lot 2; 1C) lot 3; 1D) lot 4; 1E) lot 5; 1F) lots 6, 7 and 8.

Figure 2 shows the patterns of the external controls, i.e., the undesirable background elements that were identified as components of the vaccine since they reacted with the hyperimmune serum produced in animals immunized with the vaccine of the Fuenzalida-Palacios type. Three external control elements were chosen: 2A) commercially acquired sphingomyelin, which measures the presence of antibodies against nervous tissue myelin from mouse brain; 2B) fraction V of bovine serum albumin (also a commercial product) is a very good marker for demonstrating the albumin concentration existing in the vaccines produced since its design is well defined and its electrophoretic migration is well separated from the viral fractions and from myelin, which migrate

in the same region. In addition, by measuring the area of this curve (planimetry), it is possible to establish albumin concentration; 2C) the figure also shows how the hyperimmune serum reacts with the serum fractions of mouse blood. These fractions have been previously identified as immunogenic fraction of the Fuenzalida-Palacios vaccine by NOGUEIRA & MONTAÑO[13] and were used in the present study as markers for analysis of the profiles of the various vaccines analyzed.
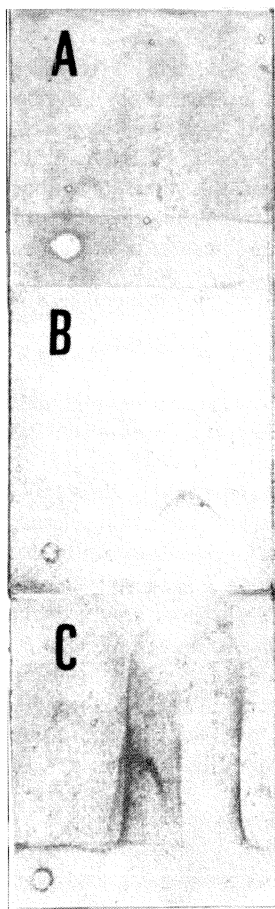

Fig. 2 - Crossed immunoelectrophoresis with hyperimmune antirabies serum incorporated into agarose gel (1:10, v/v) reacting with 5µml of the following external controls: 2a) sphingomyelin (1 mg/ml); 2b) albumin (fraction V) (1 mg/ml); 2c) mouse serum at 1:5 dilution.

Figure 3 shows the electrophoretic patterns of the various vaccines on polyacrylamide gel. The samples were obtained from the producing laboratory or directly from the Public Health Laboratory network where the vaccines are distributed and utilized. Lanes 1 and 2 correspond to the same vaccine lot produced by Laboratory A. Lane 1 corresponds to the vaccine supplied by the producing laboratory and lane 2 corresponds to the vaccine obtained from the laboratory network. Lanes 3, 4 and 5 correspond to the vaccine lots produced by Laboratory B, also obtained from the

laboratory network. It can be seen that they show different protein concentrations. Lanes 6 and 7 correspond to vaccines produced abroad by Laboratories E and F, whereas lane 8 corresponds to the third Brazilian laboratory, Laboratory C, and lane 9 corresponds to the molecular weight standard. It can be seen that the various samples presented different protein concentrations, even those belonging to the same vaccine lot (Laboratory A). This was not the case for Laboratory B since the samples, even though they were collected from the same site on the same day, did not carry a lot number on the vials.
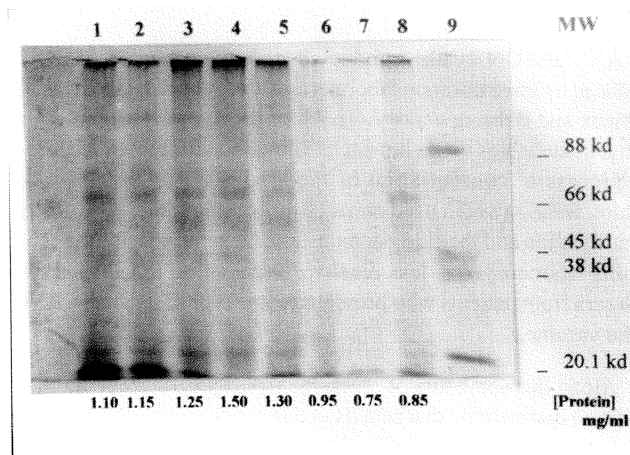

Fig. 3 - Polyacrylamide gel electrophoresis and protein concentration (mg/ml) of the vaccine doses analyzed. **Lane 1**: lot 1 (Lab. A) [1.10]; **Lane 2**: lot 1 (Lab. A) [1.15]; **Lanes 3, 4 and 5**: lot 2 (Lab. B) [1.25; 1.50; 1.30]; **Lane 6**: lot 5 (Lab. E) [0.95]; **Lane 7**: lot 8 (Lab. F) [0.75]; **Lane 8**: lot 3 (Lab. C) [0.85]; **Lane 9**: Molecular Weight Standard (88 kd ß-galactosidase; 66 kd bovine serum albumine; 45 kd egg albumine; 38 kd lactate dehydrogenase and 20.1 kd tripsin inhibitor).

DISCUSSION

The present results show that the production of vaccines of the Fuenzalida-Palacios type manufactured in Brazil does not obey a rigid manufacturing control in terms of nonspecific components. However, in the present study we did not investigate protection against rabies virus. As can be seen in Figures 1 and 3, the different proportions of substances and the different protein concentrations found in each vaccine lot reveal to what extent these highly reactive components can be responsible for the occurrence of adverse effects of vaccination (Figure 2).

According to studies with vaccines produced from duck embryos[9] 5% of the vaccinated population presented sensitivity and allergy to the vaccine This is a high and significant rate for a population of 59,000 persons vaccinated per year.

Adverse effects were also observed with the use of antirabies vaccine produced in human diploid cell culture (HDCV)[20]. BOE

et al.[3] reported a rare case of Guillain-Barré syndrome that occurred after the use of HDCV and found immune complexes representing a secondary response to the nonspecific components of the vaccine in the serum of this patient, since immunoglobulin and complement deposits were found in nerve biopsies obtained from the patient.

HDCV contains human albumin for the stabilization of rabies virus[12], but ß-propiolactone used to inactivate rabies virus reacts with albumin altering its structure and some persons can react to this new structure of albumin, developing a type III hypersensitivity reaction.

ANDERSON et al.[1] conducted a detailed study on HDCV produced by three different laboratories (Wyeth Laboratories, Institut Merieux and Behringwerke AG). All of them used ß-propiolactone to inactivate rabies virus, but each followed a different procedure in the process of concentration of the viral mass produced by cell culture. Behringwerke used zonal ultracentrifugation in the process of purification and the study demonstrated that the vaccine produced by this laboratory was less reactive, showing no reaction in tests with sera from patients who presented some type of hypersensitivity to the vaccine.

Along this same line of research, WARRINGTON et al.[18] used ELISA to demonstrate that sera from different patients who presented a reaction after an HDCV booster had high IgG and IgE concentrations against non-viral vaccine components such as cell culture medium and ß-propiolactone associated with fetal bovine serum (FBS) and human albumin. The hypersensitivity reaction and the humoral response to the vaccine components were attributed to the vaccine in 2.06% of the patients.

In vaccines produced by cell culture, small amounts of FBS are used in the cell growth medium, but the concentration of the albumin fraction of FBS become more concentrated the purification process[14].

SOUZA[16] observed that 20% of patients submitted to first vaccination presented normal anti-mouse brain antibodies starting on the 13th day after the first dose administered and revaccinated patients presented double serum titers compared to previous values also after the 13th day, demonstrating that serum conversion occurs in relation to normal brain.

In the vaccine of the Fuenzalida-Palacios type it is possible that albumin from mouse blood, together with nervous tissue, acts as a potent adjuvant that may contribute to the immunogenic potentiation of myelin in persons at higher risk, i.e., those predisposed to hypersensitivity.

However, this vaccine is effective and is a viable immunizing agent to be produced in developing countries. On the other hand, nothing prevents modification and improvement of the production process using widely accepted purification procedures previously recommended by SIKES & LARGUI[15] who used an affinity column to eliminate the impurities of the Fuenzalida-Palacios vaccine.

The introduction of one or two additional steps in the process of vaccine production would improve the quality of the product and definitely avoid this undesirable risk posed by the vaccine. Figure 1F (1%) shows that the undesirable fractions were eliminated and that only the glycoprotein (antigenic) fraction of the rabies virus was left. What may have been the process used by this laboratory?

The methodology proposed in the present study is simple and of low cost and can be utilized in any laboratory, representing an effective control method that could be easily used to control the quality of the vaccines available at Public Health services. Thus, the use of immunizing substances with a high degree of "impurity" could be avoided, especially considering this ethically unjustifiable procedure when there are means to control and avoid these impurities and their effects.

RESUMO

Efeito Adverso versus Controle de Qualidade da vacina anti-rábica tipo Fuenzalida-Palacios.

Avaliamos os componentes da vacina anti-rábica tipo Fuenzalida-Palacios que é ainda utilizada na maioria dos países em desenvolvimento na imunização humana para o tratamento profilático. Essa vacina é feita em cérebros de camundongos neonatos diluída a 1%, apesar de ser considerada uma vacina com pouco teor de mielina, no entanto ela não é totalmente livre de mielina, bem como de outros componentes indesejáveis que podem desencadear efeitos adversos a vacinação, o efeito mais grave está relacionado com o acidente neuroparalítico pós vacinal associado a síndrome de Guillain Barré.

Neste trabalho demonstramos como as vacinas produzidas e distribuidas pelos diversos laboratórios produtores apresentam padrões diferentes de seus componentes com diversos graus de impurezas e com concentrações proteicas também variadas, demonstrando que os processos de produção podem variar em cada laboratório e que essas diferenças que poderiam ser controladas por meio de um melhor controle de qualidade podem afetar e comprometer a imunização acarretando em riscos e efeitos adversos após a utilização dessas vacinas.

Utilizamos a técnica de imunoeletroforese bidimensional para avaliar e demonstrar como é possível controlar a qualidade das vacinas produzidas e diminuir os fatores de riscos que podem estar contidos nestes imunizantes.

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