Suppressant effect of human or equine rabies immunoglobulins on the immunogenicity of post-exposure rabies vaccination under the 2-1-1 regimen: a field trial in Indonesia

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WHO’s reference protocol for post-exposure rabies vaccination advises five intramuscular injections on days 0, 3, 7, 14, and 30; in addition, rabies immunoglobulins (RIG) must be given to serious cases of exposure (grade III severity). Some studies indicate that these immunoglobulins suppress the immunogenicity of rabies vaccine when administered according to an alternative protocol of four injections (2-1-1) on days 0, 7, and 21, which was therefore not recommended for grade III exposures. To test this effect, we conducted a multicentre study in Indonesia using three groups of subjects. One group received only the Vero-cell rabies vaccine (PVRV, Verorab™, usual commercial lot) according to the 2-1-1 schedule. The second and third groups received the same schedule of PVRV, plus either equine rabies immunoglobulins (ERIG, 40IU/kg body weight) or human rabies immunoglobulins (HRIG, 20IU/kg body weight). Our results confirmed the immunoglobulin suppressant effect, which was more pronounced with human than equine immunoglobulins. In both groups receiving immunoglobulins, the seroconversion rates did not reach 100% on day 28 and the geometric mean antibody titre was lower. Thus, WHO’s recommendation in 1992 of the reference protocol plus immunoglobulins for severe cases is substantiated by these results in Indonesian subjects. If the 2-1-1 regimen is chosen by the treating physician and immunoglobulins are indicated, preference should be given to purified equine RIG, which also costs less than human RIG.

Introduction

Rabies is a lethal viral encephalitis usually transmitted by the bite or scratch of a rabid animal to humans or another animal. Two post-exposure rabies vaccination protocols are currently recommended by WHO for intramuscular (IM) administration of cell-culture rabies vaccines (1). The reference protocol advises five single doses of vaccine over a 30-day period — on days 0 (D0), 3, 7, 14, and 30. The second protocol (described as 2-1-1) advises, over a 21-day period, two doses at two separate sites on D0, followed by single doses on days 7 and 21. The combined use of specific rabies immunoglobulin (RIG) on D0 is recommended for severe cases (exposure severity, grade III). Since human RIG (HRIG) is barely affordable and often not available in developing countries, the pepsin-digested, purified equine RIG (ERIG) is attracting increased interest (2).

Compared with the reference protocol, the 2-1-1 protocol, which provides two doses of antigen at the initial step of treatment, saves one dose of vaccine and two medical visits. With simultaneous administration of RIG in the 2-1-1 protocol, however, probably owing to the early high concentration of antigen and the absence of booster injections on days 3 and 14, there was a smaller antibody response even to cell-culture, purified rabies vaccines such as PVRV (3–8). The original 1992 WHO recommendations therefore did not advocate the use of the 2-1-1 regimen for severe rabies cases when RIG was indicated (1).

This paper describes a multicentre trial which compared, under field conditions, the immunogenicity of a cell-culture rabies vaccine (PVRV)
when given alone, by the 2-1-1 protocol, and in combination with RIG. In addition, to study specifically the role of homologous versus heterologous RIG on the vaccine-induced antibody response, we compared the 2-1-1 schedule when combined with HRIG or ERIG.

Population and method

Indonesian males and females, aged 13–55 years, who had never received pre- or post-exposure rabies vaccination and who freely gave their informed consent, were enrolled between July 1993 and May 1994 into the study, which followed WHO recommendations on good clinical practice. The subjects were recruited from four centres (three in Java and one in Sumatra) and either had been exposed to rabies (and required treatment within 24 hours of exposure) or were healthy volunteers. Subjects in group A showed an exposure severity of grade I or were healthy volunteers and received the 2-1-1 PVRV treatment without rabies immunoglobulins. Subjects who were given RIG (groups B and C) had been bitten, with an exposure severity of grade II (for the purpose of this clinical trial) or grade III (by the WHO definition). All subjects in groups B and C received the same 2-1-1 vaccine treatment as group A, but also a booster vaccine injection on day 90. On day 0, group B subjects received ERIG while group C received HRIG with the vaccine injection.

A commercially available, rabies cell-culture vaccine, prepared on Vero cells (Verorab™, Pasteur Mérieux Connaught (PMC), Lyon, France) and having an antigen content greater than 2.5IU/dose (batch H0768; exact titre, 4.3IU/0.5ml) as determined by the National Institutes of Health test, was injected into the deltoid muscle. More than 10 million doses of this vaccine, first registered in 1986 and marketed in over 40 countries, have been distributed worldwide. All subjects received two 0.5-ml vaccine doses on D0 and single doses on D7 and D21. As specified in the protocol, an additional 0.5-ml dose was administered on D90 to the subjects in groups B and C. ERIG (Pasteur Rabies Serum™, PMC; commercial batch, H5717) at a titre of 200IU/ml (as determined by the mouse neutralization test) was used at the WHO-recommended dose of 40IU/kg. HRIG (Imogam™ Rabies, commercial batch J0106, PMC) with a titre of 150IU/ml (as determined by the rapid fluorescent focus test, RFFIT) was given at the WHO-recommended dose of 20IU/kg. On D0, half of the RIG dose was instilled deep into the wound to infiltrate into the adjacent area, and the other half was injected IM into the buttock. At the time of this trial, a skin test for sensitivity to equine RIG was performed prior to administration of ERIG (1).

Venous blood samples were obtained from all subjects prior to vaccinations on D0, D28, and D90, and on D97 from groups B and C. The blood samples were immediately centrifuged, and the sera were frozen (−20°C). Immunogenicity, evaluated by a neutralizing antibody RFFIT assay, was performed at PMC (Val de Reuil, France). An elevated antibody titre (≥0.5IU/ml) confirmed seroconversion and was considered to be protective (8).

A minimum of 59 subjects had to be included in each group so that the lower limit of confidence for seroconversion would be >95% if a 100% seroconversion was observed in the study population. Each subject given only PVRV (group A) was paired, according to age and sex, with a PVRV + ERIG subject (group B) and a PVRV + HRIG subject (group C). Two assessment criteria were used. Seroconversion, expressed in terms of a seroconversion rate (SCR), was the qualitative criterion and was considered to have been reached when the antibody titre was ≥0.5IU/ml. The quantitative criterion was the amplitude of the antibody response, as expressed by the geometric mean titre (GMT). Fisher’s exact test was used to verify homogeneity between groups by age and sex, analysis of variance (Duncan’s test) to compare groups for seroconversion, and the χ²-test to compare groups for GMT after logarithmic conversion of the titre values (Statistical Analysis System, version 6.04). A difference between groups was considered to exist for P < 0.05.

Results

A total of 241 subjects, 111 women (46.1%) and 130 men, were enrolled in the study. The mean age was 29.3 years (range, 13–58 years; SD, 12.4 years) and did not differ significantly between the three groups. Deviations from the protocols and dropouts led to the final analysis of only 177 subjects on D0 (group A = 73, B = 43, and C = 61), 151 subjects on D28 (group A = 61, B = 36, and C = 54), and 136 subjects on D90 (group A = 58, B = 33, and C = 45). Although safety was not specifically studied, no serious reactions occurred in any group during the trial period.

No subject had seroconverted at the 0.5IU/ml level on D0. The seroconversion rates (SCR) for D28 and D90 are summarized in Table 1. A significant difference between groups (P = 0.03) was noted on D28 when the SCR for group A was 100%; that for group B (94.4%) was greater than that for group
C (90.7%). On D90, statistical comparisons were not performed because of the small number of subjects in any group. None of the groups showed 100% SCR on D90.

Table 2 presents the GMT values obtained for each group on D0, D28, D90, and D97. A significant difference between groups was noted on D28 by the χ²-test. Although Duncan’s test showed no significant difference either between groups A and B or between groups B and C, the antibody titres were significantly higher in group A than in group C. This significant difference persisted on D90.

Discussion

Our aim was to compare the immunosuppressant effect of rabies immunoglobulin of equine or human origin on the WHO-approved alternative 2-1-1 protocol for rabies post-exposure prophylaxis. The results show a suppressive effect of RIG on vaccine immunogenicity, the seroconversion rates and GMT values being significantly lower in the group receiving human RIG than equine RIG. Owing to logistical constraints in the field, such as non-randomized assignment of ERIG and HRIG, differences in the numbers of patients in each group, and imperfect pairing because of unequal group sizes, any definite conclusions should be made cautiously.

On day 90, none of the groups had achieved 100% seroconversion, but the GMT of those who received human RIG (group C) was still significantly lower than for the subjects without RIG (group A). Our results thus confirm previous reports (3–7), which showed a decreased neutralizing antibody

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<td>(90.8–100)</td>
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* Statistically significant difference between these groups (Duncan’s test).
* Figures in parentheses are 95% confidence intervals.

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* Statistically significant difference between these groups χ²-test.
* ND: not done.
response to the 2-1-1 post-exposure vaccination protocol, from D10 to D90, as a result of simultaneous RIG administration. This effect of RIG may have prevented seroconversion from consistently reaching 100%, as was the case in our study on day 28, and also consistently and significantly decreased the GMTs up to day 90 in our study.

This decrease in GMT values was more pronounced in intensity and duration for human than for equine RIG, probably because of the longer half-life of homologous HRIG (about 21 days) compared to heterologous ERIG (about 3 days). Experimentally, after administration of homologous antirabies serum the circulating antibody level decreased more slowly than after heterologous antiserum, and the response to simultaneously administered rabies vaccines was suppressed and delayed longer by homologous antiserum than by heterologous antiserum (9). The relationship between the half-life of the RIG and its suppressant effect on vaccine has been shown using monoclonal antibodies in a mouse model (10). In animals, this suppressive effect induces susceptibility to challenge with rabies viruses (11).

Such susceptibility depends also on the potency of the vaccine that is co-administered. We therefore chose to give the usual, commercially available vaccine in Indonesia to mimic "real life" conditions. Unlike our results, the use of an experimental cell-culture vaccine lot with a high antigenic content has been shown to decrease the interaction with human RIG, as reported recently (12). In other published comparative trials, a 2-1-1 protocol that did not include administration of RIG enabled 100% seroconversion to be obtained on day 28, and the GMTs were of the same magnitude as those obtained with the reference protocol using five vaccine injections (3–7). In addition, in Thai children (aged 7–13 years) the SCR values at 3, 6, and 12 months after the 2-1-1 regimen alone were, respectively, 100%, 89%, and 92%, but only 76%, 37%, and 13% after inclusion of HRIG (13).

Finally, although some of our patients did not reach the arbitrary WHO-approved seroconversion level of 0.5IU/ml, we should not assume that they were not protected against rabies. For example, Chutivongse et al., using the 2-1-1 PVRV regimen + equine RIG, reported that 5 out of 10 subjects had an antibody level <0.5IU/ml and, despite that, all 100 subjects bitten by rabies-proven dogs were still alive after one year (6).

In conclusion, the seroconversion results from our field study support WHO's recommendation to use five injections on days 0, 3, 7, 14 and 30, particularly in severe cases who require the administration of RIG. However, in agreement with a WHO Expert Committee's recommendation made in 1997 (14), if the 2-1-1 protocol is nevertheless chosen by a physician to treat severe rabies exposure, preference should be given to administration of purified equine RIG, which also costs less than human RIG.

Acknowledgements

We are indebted to Dr L. Teulières, Dr H. Debois, and Dr I. Gusmari for making this study possible. We also thank the following for their contributions: Mr P. Brantais (statistics), Mrs. C. Blondeau (RFFIT assays), I. Furman and M. Fletcher (editorial services), V. Pichon (monitoring), M. Beigeaud and F. Kahn (secretarial support), and Dr A. Strady for excellent advice during the writing of this manuscript.

Résumé

Effet inhibiteur des immunoglobulines antirabiques humaines et équines sur l’immunogénicité de la vaccination antirabique curative utilisant le protocole 2-1-1: essai sur le terrain en Indonésie

Le protocole de référence de l’OMS pour la vaccination antirabique curative recommande cinq injections intramusculaires aux jours 0, 3, 7, 14 et 30; en outre, des immunoglobulines antirabiques doivent être administrées en cas d’exposition grave (gravité de niveau III). D’après plusieurs études, ces immunoglobulines inhiberaient l’immunogénicité du vaccin antirabique lorsque celui-ci est administré suivant un schéma comportant quatre injections (2-1-1) aux jours 0, 7 et 21; il n’est donc pas recommandé quand l’exposition est de niveau III. Pour vérifier l’existence d’un tel effet, nous avons mené en Indonésie une étude multicentrique sur trois groupes de sujets. Le premier groupe n’a reçu que le vaccin antirabique préparé sur cellules Vero (vaccin antirabique préparé sur cellules Vero purifiées, Verorab, lot commercial courant) conformément au protocole 2-1-1. Le deuxième et le troisième groupe ont été traités suivant le même protocole, avec le vaccin antirabique produit sur cellules Vero et purifié, associé à des immunoglobulines antirabiques d’origine équine (40UI/kg de poids corporel) ou humaine (20UI/kg de poids corporel). Nos résultats confirment l’effet inhibiteur des immunoglobulines, plus marqué avec les immunoglobulines humaines qu’avec
les immunoglobulines équines. Dans les deux groupes traités par des immunoglobulines, le taux de séroconversion n'a pas atteint 100% au jour 28 et la moyenne géométrique des titres d'anticorps est inférieure. Les recommandations de l'OMS de 1992 s'appliquant aux cas graves et associant vaccination conforme au protocole de référence et immunoglobulines sont donc justifiées par les résultats obtenus chez les sujets indonésiens. Si le médecin traitant choisit le protocole 2-1-1 et si les immunoglobulines sont indiquées, la préférence doit être donnée aux immunoglobulines antirabiques équines purifiées, qui ont aussi l'avantage d'être moins onéreuses que les immunoglobulines antirabiques humaines.

References