REPORT

WORKING GROUP ON
JAPANESE ENCEPHALITIS VACCINES

Osaka, Japan
5-7 February 1987

Manila, Philippines
November 1987
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NOTE

The views expressed in this report are those of the members of the Working Group on Japanese Encephalitis Vaccines and do not necessarily reflect the policies of the World Health Organization.

This report has been prepared by the Regional Office for the Western Pacific of the World Health Organization for governments of Member States in the Region and for those who participated in the Working Group on Japanese Encephalitis Vaccines which was held in Osaka, Japan, from 5 to 7 February 1987.
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1. INTRODUCTION

A meeting of the Working Group on Japanese Encephalitis Vaccines was held in Osaka, Japan, on 5-7 February 1987. The meeting was convened by Dr Hiroshi Nakajima, Regional Director, Regional Office for the Western Pacific, who expressed his gratitude to the Government of Japan and the Osaka University Research Foundation for Microbial Diseases for their assistance in organizing the meeting.

Japanese encephalitis is of increasing concern in the South-East Asia and Western Pacific Regions. The disease has continued to cause high morbidity and mortality and was especially severe in 1985 in Viet Nam. The WHO Regional Office for the Western Pacific has responded to requests for vaccines, drugs and insecticides for mosquito control and has already held meetings on the prevention and control of Japanese encephalitis, vaccine development and vaccine strategies. A meeting of a scientific group on development of recombinant DNA Japanese encephalitis and dengue vaccines immediately preceded this working group. The meetings held have resulted in an active interchange leading to the improvement of vaccines; however, the products available are still too expensive for mass utilization. In view of the need to formulate draft WHO requirements for Japanese encephalitis inactivated vaccines for human use, so that they can be made more widely available, the present working group was convened. Its terms of reference were:

1. to review the progress of Japanese encephalitis vaccine development;
2. to assess the quality of inactivated Japanese encephalitis vaccine; and
3. to formulate WHO requirements for inactivated Japanese encephalitis vaccines.

The Regional Director expressed the hope that the final outcome would contribute to a strategy for the prevention and control of Japanese encephalitis in the South-East Asia and Western Pacific Regions.

2. JAPANESE ENCEPHALITIS VACCINE DEVELOPMENT

2.1 Field trials of inactivated mouse-brain Japanese encephalitis vaccine

A field trial of inactivated mouse-brain Japanese encephalitis vaccine was carried out in Thailand between January and March 1985. The trial took place in Kamphaeng Phet Province of northern Thailand, where a total of 73,555 children received monovalent Nakayama Japanese encephalitis vaccine, bivalent Nakayama-Beijing Japanese encephalitis vaccine or control tetanus toxoid in three different groups in a blind placebo control study. The vaccines were administered by a jet injector apparatus in 458 schools in seven districts.

There was a single fatality from Reyes syndrome, a child in the tetanus toxoid group. Otherwise, side effects were minimal and did not differ among the groups.
Blood samples were taken from first ten vaccinated in each school before and one month after vaccination, and 1127 samples were tested for neutralizing antibody to the Nakayama strain of the Japanese encephalitis virus. Japanese encephalitis is endemic in Kamphaeng Phet Province, and preexisting Japanese encephalitis antibody was found in 5% of children aged one year and in over 90% of children aged 14 years. After vaccination, 58% of the one-year-old cohort developed neutralizing antibody in the monovalent Japanese encephalitis group and 50% in the bivalent Japanese encephalitis vaccine group. The 14-year old cohort had 95% and 96% neutralizing antibody, respectively, when tested after vaccination. The log geometric mean titre (GMT) of Japanese encephalitis neutralizing antibody rose in recipients of the Japanese encephalitis vaccine, whether antibody had already been present or not.

There was no significant difference in antibody response between the monovalent and bivalent groups; however, it was discovered after the trial that the Beijing component of the bivalent vaccine had lost potency between the time of manufacture and the field trial. Thus, the two Japanese encephalitis vaccine groups could be assumed to have received antigenically comparable vaccines.

Surveillance was maintained for two epidemic seasons during which no further Japanese encephalitis vaccine was given. The attack rate in the placebo group was 41.9 per 100 000 in 1985 and 13.9 per 100 000 in 1986. There was a single confirmed case of Japanese encephalitis in the bivalent group during 1985. The patient survived. There was a single fatal case in the monovalent group in 1986. The overall attack rate in the two Japanese encephalitis vaccine groups was 4.6 per 100 000, and the overall efficacy rate was 92%. On the basis of sero-conversions in the placebo group, it was estimated that the infection rate was 7700 per 100 000. There were 42 cases of Japanese encephalitis per 100 000, giving a 183:1 inapparent:apparent infection ratio.

IgM capture ELISA was carried out on 1858 children, from whom blood samples were taken following a febrile illness with headache and other central nervous system symptoms during the 1985 and 1986 epidemic seasons. Twenty-seven were positive for Japanese encephalitis IgM and were thus classified as presumptive mild Japanese encephalitis cases.

Dengue fever and dengue haemorrhagic fever attack rates were lower among recipients of Japanese encephalitis vaccine than in the placebo group, but the difference was significant at \( p < 0.05 \) only during a brief period immediately following vaccination in 1985.

The manufacturing process of the Beijing-1 mouse-brain vaccine was refined to improve product stability. A field trial of this vaccine in 121 children in Japan gave sero-conversion rates as measured by the Beijing-1 virus of 97.6% after two doses and 100% after three doses. The rates as measured by the Nakayama neutralization test were slightly lower and titres were also lower. After booster doses one year later, a rise in titre could be demonstrated for both Beijing-1 and Nakayama viruses. The Beijing-1 Japanese encephalitis vaccine will be available in 1989.
2.2 Review of efficacy of inactivated hamster-kidney Japanese encephalitis vaccine

The Working Group reviewed efficacy trial results accumulated over the past 18 years. The vaccine is produced in primary hamster-kidney cells infected with the P-3 strain. The formalin-inactivated product produced a 100% response in guinea pigs and horses.

A field trial was carried out between 1973 and 1978 in Jilin Province, China, where the pre-vaccination antibody rate in 334 children was 1.4%. The sero-conversion was 60% after the primary series and 93% after a booster dose one year later. This rate diminished to 64% over an additional four years but rose to 100% after a second booster dose.

Field trials were carried out in five provinces of China, between 1967 and 1973. About 300 000 children were vaccinated in Wuxi (1967), Nanjing (1968), Beijing (1969), Hunan (1968) and Guangxi (1973) provinces with more than 180 000 children serving as controls. The incidence of Japanese encephalitis in the vaccinated groups was reduced 4 to 20 times; the protective rate was 76% to 94%. In addition, studies in Wuxi and Nanjing indicated that the illnesses occurring in vaccinated children were milder than in unvaccinated children.

Mass vaccination of children under 15 years in Beijing has resulted in about 85% coverage. The age distribution of cases shifted from 77% of total cases in the one- to nine-year old group in 1963 to only 36% in 1979. The incidence dropped from 25 per 100 000 in 1966 to less than 0.3 per 100 000 in 1985.

The hamster-kidney vaccine has been concentrated by two ultrafiltration methods. Both the hollow fibre and the pellicon cassette systems have resulted in a more than tenfold concentration and improvement in potency.

2.3 Studies of Japanese encephalitis attenuated-virus vaccines

Four lots of an attenuated Japanese encephalitis virus vaccine (SA14-14-2) were prepared and safety-tested for use in humans. More than 1000 children between the ages of 5 and 12 years, living in an area of low Japanese encephalitis infection, were vaccinated with one of the four lots of the vaccine. A selected group of 47 vaccinated children were examined for fever and other systemic reactions every other day for two weeks following vaccination. None of these children had fever (temperature over 37.4°C) or other systemic reactions during the observation period. No untoward reactions were reported in the remainder of those vaccinated. After immunization, sero-conversion rates in seronegative children were 100% (GMT 35.3, n=11), 100% (GMT 31.7, n=12) and 83.3% (GMT 23.0, n=10) in groups receiving vaccine diluted 1:3 (6.9 log 10 TCID50), 1:5 and 1:50, respectively. These results indicate that the Japanese encephalitis attenuated vaccine virus is immunogenic, apparently phenotypically stable, and safe for children.
The SA14-14-2 candidate vaccine was pathogenic for BALBc-nu/nu mice by intracutaneous (i.c.) inoculation, but the incubation period was prolonged in comparison with the parent virus. SA14-14-2 virus was not pathogenic for nude mice subcutaneously (s.c.) or intraperitoneally (i.p.). The candidate vaccine differed in its oligonucleotide fingerprint pattern from the parent and had a very broad antigenic coverage in mice vaccinated with SA14-14-2 virus and challenged i.p. with 10 different Japanese encephalitis virus strains.

Two other attenuated Japanese encephalitis strains, SA14-2-8 and SA14-5-3, were used to immunize mice in parallel with an inactivated Japanese encephalitis vaccine. The mice were then immunosuppressed with cyclophosphamide and all three groups were challenged with Beijing-1 virulent virus. The groups immunized with attenuated virus resisted challenge, whereas 8/10 mice immunized with inactivated vaccine succumbed.

3. QUALITY CONTROL OF INACTIVATED VACCINES

3.1 Determination of protein content (viral antigen and myelin basic protein) in mouse-brain preparations of Japanese encephalitis vaccine

Viral antigen in a mouse-brain Japanese encephalitis vaccine preparation was assayed by quantitative immune precipitation with radiolabelled anti-Japanese encephalitis mouse immunoglobulin. The total protein content of the sample was determined and the amount of radioisotope from precipitated antibody-antigen complexes was counted in order to calculate the concentration of viral antigen. Results from these assays showed that the average viral antigen content of a Japanese encephalitis vaccine was 98%.

In order to assay the amount of myelin basic protein, a double antibody competitive radio-immuno-assay was used. One vaccine lot had 2ng/ml; the rest had less than 2ng/ml myelin basic protein. One ng/ml is the minimum amount of myelin basic protein required to induce histological change in Hartley strain guinea pigs.

3.2 Stability of inactivated mouse-brain Japanese encephalitis vaccine

The stability of inactivated mouse-brain Japanese encephalitis vaccine in the liquid state was measured at 10°C, 25°C and 37°C. Potency was determined by the mouse antibody production test with plaque reduction neutralization antibody assay. The vaccine held at 10°C retained its potency for 26 months or more. Vaccine held at 25°C gradually lost potency, but was satisfactory for 12 weeks. The vaccine held at 37°C was satisfactory for one week but not longer.

3.3 Protein determination

The Lowry method to determine TCA-precipitable protein content of mouse-brain Japanese encephalitis vaccine has been substituted in Japan for the micro-Kjeldahl. The Lowry method is more sensitive at levels of protein under 10 micrograms of N per ml.
3.4 Potency test

The antibody production potency test was compared to the vaccination-challenge test. The antibody production test was found to correlate with sero-conversion rates in vaccinated children, whereas the vaccination-challenge results correlated when hamster-kidney vaccine was tested, but not when mouse-brain vaccine was tested. Chicken-embryo tissue cultures were compared with BHK-21 cell cultures in the plaque reduction neutralization test as part of the antibody-production potency test. The results of the two methods were comparable.

3.5 Collaborative study of the potency test

At least four laboratories agreed to participate in a collaborative study of the potency test for inactivated Japanese encephalitis vaccine. Reference antibodies to the Nakayama-NIH and the P-3 strains of Japanese encephalitis virus have been prepared. The Working Group reviewed and agreed upon the following protocol:

1. Six vaccines will be tested: Nakayama and Beijing-1 lots from Japan, two Nakayama lots from the Republic of Korea and two P3 lots from China.

2. Ten four-week-old mice will be immunized with vaccine dilutions 1:4, 1:6 and 1:64 on days 0 and 7. Serum collected on day 14 will be pooled and tested in the plaque reduction neutralization (PRN) test. Sera from the 10 mock-inoculated mice will be included as a negative control.

3. PRN tests will be carried out in chicken-embryo and BHK-21 cells with Nakayama-NIH, Beijing-1 and P-3 viruses.

4. Results will be reported for comparative analysis to the WHO Collaborating Centre for Arbovirus Research and Reference, National Institute of Health (NIH), Tokyo.

4. DRAFT REQUIREMENTS FOR INACTIVATED JAPANESE ENCEPHALITIS VACCINES

The Working Group drafted requirements for inactivated Japanese encephalitis vaccines. There was agreement in principle that the bulk vaccine should be tested for residual infectious virus after inactivation, and that a substantial amount (at least 25 human doses) of vaccine should be assayed. The draft document will be submitted to WHO with the request that the requirements be considered by the Expert Committee on Biological Standardization.
5. RECOMMENDATIONS

(1) The draft requirements for Japanese encephalitis vaccine which were developed at this meeting should be evaluated further by WHO so that they can be considered by the Expert Committee on Biological Standardization.

(2) WHO should support the transfer of technology necessary to conduct the Japanese encephalitis antibody production potency test.

AGENDA

(1) Registration

(2) Opening ceremony

(3) Field trial of bivalent and monovalent inactivated JE vaccines in Thailand
   Dr N. Sangkawibha

(4) Field trial of bivalent and monovalent inactivated JE vaccines in Japan
   Dr T. Kitano

(5) New development of criteria on JE vaccine requirement in Japan
   Dr A. Oya

(6) Quality control of vaccine in China
   Dr Li He-min

(7) Quality control of vaccine in the Republic of Korea
   Ms H.W. Cho

(8) Standardization of JE vaccine potency test
   Dr R. Shope

(9) Formulation of WHO requirements for inactivated Japanese encephalitis vaccines
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