Safety and immunogenicity of a Vero-cell-derived, inactivated Japanese encephalitis vaccine: a non-inferiority, phase III, randomised controlled trial

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Summary

Background **Japanese encephalitis virus (JEV) is the leading cause of viral encephalitis in southeast Asia. Although no treatment is currently available, vaccination effectively prevents the disease. In a non-inferiority study, we aimed to compare the safety and immunogenicity of a novel, second-generation, inactivated candidate vaccine for JEV with a licensed, mouse-brain-derived vaccine.**

Methods **We included 867 adults in a multicentre, multinational, observer-blinded, randomised controlled phase III trial. Study sites were located in the USA, Germany, and Austria. Volunteers received either the JEV test vaccine intramuscularly on a two-dose schedule (on days 0 and 28; n=430) or the licensed vaccine subcutaneously according to its recommended three-dose schedule (on days 0, 7, and 28; n=437). The primary endpoint was immunogenicity, with respect to neutralising JEV-specific antibodies assessed by a plaque-reduction neutralisation test, which was assessable in 725 patients in the per-protocol population. This trial is registered as a clinical trial, EudraCT number 2004-002474-36.**

Findings **The safety profile of the test vaccine was good, and its local tolerability profile was more favourable than that of the licensed vaccine. Frequency of adverse events was similar between treatment groups, and vaccine-related adverse events were generally mild. The seroconversion rate of the test vaccine was 98% compared with 95% for the licensed vaccine on day 56 (95% CI for the difference –1·33 to 3·43). Geometric mean titre for recipients of the test vaccine was 244 (range 5–19783), compared with 102 (5–1864) for the licensed vaccine (ratio 2·3 [95% CI 1·967–2·75]).**

Interpretation **The test JEV vaccine has a promising immunogenicity and safety profile.**

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Introduction

Japanese encephalitis is the most important cause of viral encephalitis in Asia. The Japanese encephalitis virus (JEV) is estimated to cause at least 50 000 cases of clinical disease every year, mostly in children younger than 10 years.[1,2] The disease is endemic in southeast Asia, a region with more than 3 billion inhabitants. Recently, within only 1 month, Japanese encephalitis killed more than 1200 children during an epidemic outbreak in Uttar Pradesh, India, and Nepal.[3]

Vaccines against this mosquito-borne, flavivirus infection were developed in Russia and Japan as early as the 1930s. Several first-generation, inactivated vaccines, with mouse brain as a substrate for growth of the virus, have been produced by Japanese, Korean, Vietnamese, and other national manufacturers for decades. More recently, Chinese manufacturers have produced both inactivated and live virus vaccines using predominantly a primary hamster-cell line for virus propagation.[4–7]

Only one vaccine for Japanese encephalitis is licensed in the USA, Canada, and Australia, but none is licensed in Europe (JE-VAX, Foundation for Microbial Diseases, Osaka University, Biken, Japan). This formalin-inactivated, mouse-brain-derived vaccine is manufactured by the Foundation for Microbial Diseases of Osaka University (Biken) in Japan. The protective efficacy of the licensed vaccine was shown to be 91% in about 65 000 children in Thailand.[8] Serious side-effects, such as anaphylaxis occurring typically 1–3 days (up to 17 days) after vaccination, have been noted with an occurrence of 15–62 per 10 000 people in the USA. Although the exact cause of these reactions is unknown, most experts regard the porcine gelatin stabilisers included in the formulation of the licensed vaccine to be responsible for these severe side-effects. Furthermore, neural tissue content of the vaccine has raised concerns about safety and the possibility of vaccine-related neurological side-effects.[9] These safety concerns led to the suspension of routine vaccination with mouse-brain-derived inactivated JEV vaccines in Japan in May, 2005.[10]

The manufacturer of the licensed vaccine has reported that its production for use in developed countries has been discontinued and existing supplies will probably be exhausted in the next few years. Live, attenuated vaccines are available in certain endemic, developing countries, but will probably not be licensed in developed countries. Also, a chimeric, live, attenuated vaccine is currently undergoing development.[11] Here, we report phase III clinical data of a novel, purified, inactivated JEV vaccine using a certified Vero-cell culture substrate for virus propagation.

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Methods

Participants

In this multicentre, observer-blinded, centrally randomised controlled trial, the study population consisted of healthy male and female volunteers, aged at least 18 years. Of 867 people who were randomly assigned vaccines, two dropped out because of adverse events before the first immunisation, one decided to withdraw his consent, and another did not complete the visit. Thus, 863 people actually received study medication between Sept 5, 2005, and March 17, 2006 (662 vaccinated at eight study sites in the USA, and 201 vaccinated at three sites in Austria and Germany). All US sites and the site in Berlin, Germany, were professional site-management organisations for clinical trials. Both Austrian sites were at the Medical University of Vienna. Most sites recruited participants after screening their client files, but also after news and radio advertisements, which had been approved by independent review boards and ethics committees.

Participants were enrolled into the trial by masked principal investigators. A centralised, computerised procedure (interactive voice response service [IVRS]) was used to randomly assign participants by age, sex, and study site. An independent statistician had the sole role of providing the randomisation code before the study began. Treatment allocation was reported to a centralised logistics centre for providing study medication, as well as to the unmasked investigators who gave the vaccine. Allocation concealment was done via IVRS (an unmasked staff member with no other duties received the box number after entry of patient details during a telephone call).

We enrolled only healthy volunteers who gave written informed consent. Exclusion criteria consisted of previous flavivirus infection, previous immunisation for Japanese encephalitis or yellow fever, use of any investigational or non-registered drug or other vaccine during the study period or within 30 days preceding the first dose of study vaccine, use of immunosuppressants or other immune-modifying drugs within 6 months of vaccination, use of any other vaccine during the study, and previous history of severe hypersensitivity reactions. Most participants who decided not to continue with the study withdrew their consent for personal reasons. Adverse events rarely led to withdrawal. Most volunteers who dropped out of the per-protocol population did so because of seropositivity of Japanese encephalitis at baseline, major protocol violations, or discontinuation of the study (figure 1). The study protocol was approved by independent review boards and the US Food and Drug Administration (FDA), and by the ethics committees and national health authorities in Austria and Germany.

Procedures

The JEV test vaccine (Intercell Biomedical, Livingston, UK) is a purified inactivated vaccine, containing the JEV strain SA_{14}-14-2. This attenuated strain was adapted to grow in Vero cells. The vaccine was prepared by a purification and inactivation process consistent with current good manufacturing practices. The finished product does not contain thimerosal or gelatins. One vaccine dose contained 6 μg of purified and inactivated virus adsorbed to 0·1% aluminum hydroxide. 0·5 mL of the test vaccine was injected into the deltoid muscle of participants on days 0 and 28. 1 mL of the licensed vaccine was injected subcutaneously on days 0, 7, and 28 into the upper arm. To mask the comparator, the group receiving the test vaccine also received a placebo shot (with 0·5 mL of 0·1% alum diluent) on day 7, thus providing identical injection schedules for both groups.

The licensed vaccine is available as lyophilised powder in vials, which needs to be reconstituted and given subcutaneously. The test vaccine, by contrast, comes in liquid formulation in ready-to-use prefilled syringes. To overcome this potential limitation, all investigational sites nominated a masked and an unmasked investigator. The vaccines were prepared and injected by an unmasked staff member at the site (who did not subsequently participate in the study), whereas the participants and all other investigators and staff members remained masked throughout the trial. The primary aim of the study was to investigate the immunogenicity of the test vaccine compared with the licensed vaccine in terms of seroconversion rates and geometric mean titres of JEV-neutralising antibody at 4 weeks after the last vaccine dose (day 56). The secondary aim was to assess and compare the safety of the two vaccines.

Adverse events after immunisation were recorded by participants in a diary, starting on the day of every vaccination and continuing for 7 consecutive days. Intensity grades were given to local injection site redness, swelling, and fever. Local tolerability was also assessed by a masked examiner. Systemic tolerability, as well as vital signs and major blood analyses, were reviewed by investigators at every visit. A placebo was used as the second injection for the test vaccine group. Therefore, for a vaccine-to-vaccine comparison, injections 1 and 3 on days 0 and 28, respectively, in the test vaccine group should be compared with all three doses in the licensed vaccine group. Furthermore, a comparison of injections 1 and 3 versus injection 2 on day 7 in the test vaccine group compares safety between the test vaccine and placebo.

For immunogenicity, JEV-specific neutralising antibodies, as measured by the plaque-reduction neutralisation test (PRNT), provide a reasonable immune correlate of protection.[12] Seroconversion is commonly defined as a $PRNT_{50}$ titre (serum dilution giving a 50% plaque reduction compared with plaque formation in virus-only controls) of at least 1:10, which is a cut-off established by animal experiments.[13] Correlation with protective efficacy has been shown in the phase III study in Thailand supporting the licensing of JE-VAX in the USA.[8]

Serum samples were taken from participants before vaccination on days 0, 28, and 56. Samples were stored

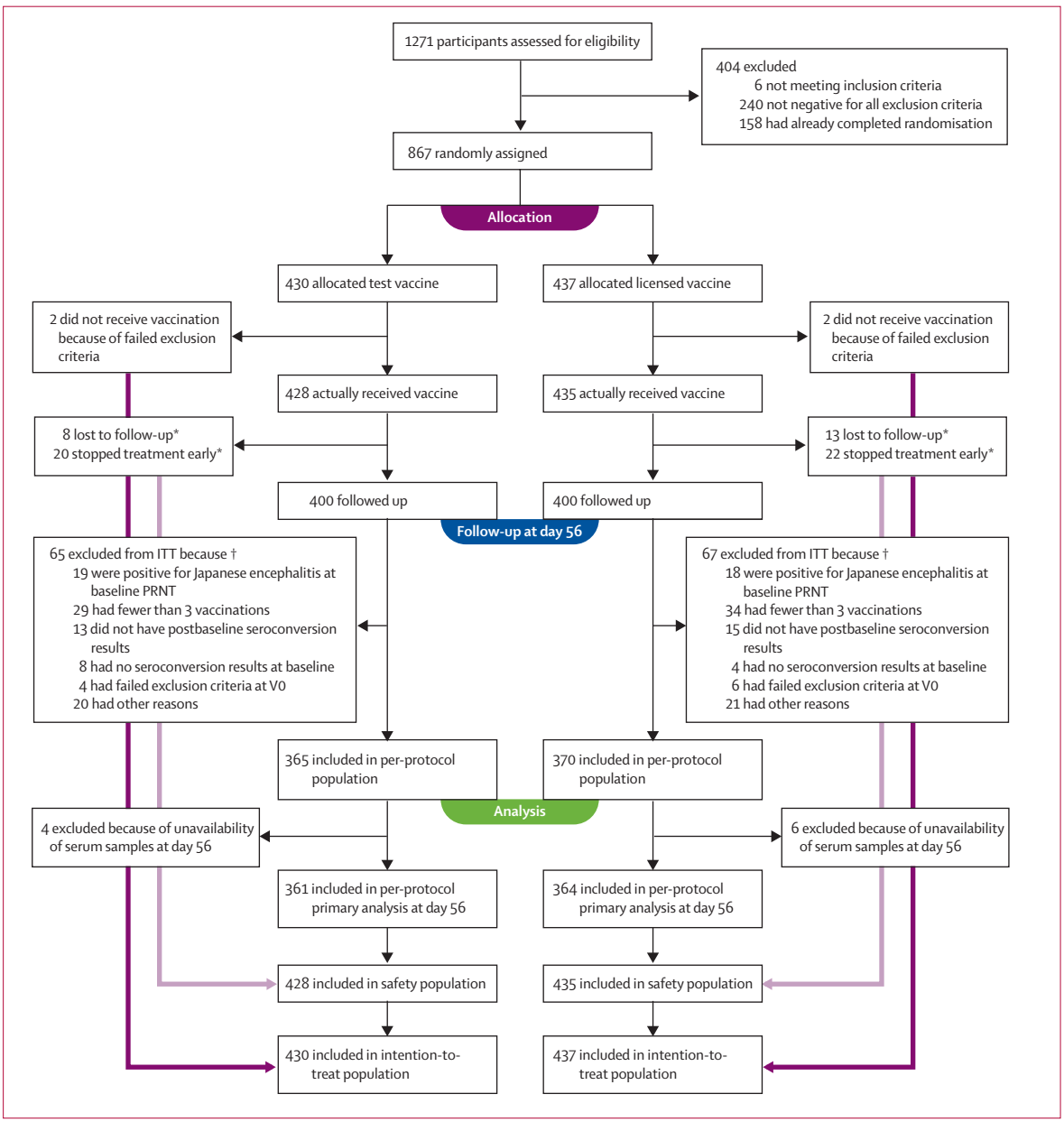


Figure 1: **Trial profile**
Randomisation already completed indicates that recruitment was stopped because the necessary number of participants had already been randomly assigned. Not negative for all exclusion criteria indicates that at least one exclusion criterion was met. V0=visit 0 (ie, screening visit). *Included in intention-to-treat (ITT) and safety populations, but not in per-protocol (PP) population. †More than one reason could apply.

at –80°C and shipped on dry ice. All analyses were done under good laboratory practice with a validated PRNT[14] in a central laboratory at Intercell AG, Vienna, Austria. Briefly, serial dilutions of test serum (1:10, 1:40, 1:160, 1:640, or higher if needed) were incubated for 1 h at 35°C with a defined number of JEV-plaque-forming units (400 pfu/mL) and plated in triplicate onto a monolayer of Vero cells with a methylcellulose overlay to restrict virus spread. After 5 days of incubation (35°C, 5% CO_2, saturated H_2O), the viral plaques were fixed, stained with crystal violet, and automatically counted (ProtoCOL HR Colony Counter, Synbiosis, Cambridge, UK).

We calculated $PRNT_{50}$ titres using a linear regression (PROBIT) analysis programme. In a dose-response analysis, the PROBIT model transforms a sigmoid-shaped observed response proportion into a linear-shaped response variable. All analyses were done with the test vaccine strain (SA_{14}-14-2) for PRNT, because of biohazard precautions but also for standardisation reasons. A potential bias in favour of the experimental vaccine was

	Test vaccine (n=428)	Licensed vaccine (n=435)	Overall (n=863)
US sites (n)	329	333	662
Europe sites (n)	99	102	201
Median age (range [years])	41 (18–79)	41 (18–80)	41 (18–80)
Mean age (SD [years])	41·6 (15)	41·1 (14)	41·3 (14)
Median height (range [cm])	170 (130–198)	170 (144–197)	170 (130–198)
Median weight (range [kg])	77 (42–170)	77 (43–189)	77 (42–189)
Median body-mass index (range [kg/m^2])	26 (16–56)	26 (16–69)	26 (16–69)
US site median age (range [years])	43 (18–79)	43 (18–80)	43 (18–80)
European site median age (range [years])	33 (18–68)	34 (18–66)	33 (18–68)
Male (n [%])	161 (38%)	177 (41%)	338 (39%)
Female (n [%])	267 (62%)	258 (59%)	525 (61%)
Ethnic origin (n [%])			
White	338 (79%)	359 (83%)	697 (81%)
Black	59 (14%)	54 (12%)	113 (13%)
Asian	5 (1%)	2	7 (1%)
Other	26 (6%)	20 (5%)	46 (5%)
Participants positive for Japanese encephalitis in baseline PRNT	19 (4%)	18 (4%)	37 (4%)

Table 1: **Demographic characteristics of safety population (n=863)**

	Test vaccine	Licensed vaccine	Risk difference estimator (95% CI)*
Seroconversion (%)	98% (352/361)	95% (347/364)	1·05 (–1·33 to 3·43)
Geometric mean titre (range)	244 (5 to 19 783)	102 (5 to 1864)	2·3 (1·967 to 2·75)

*Mantel-Haenszel risk difference estimator for seroconversion (test vaccine minus licensed vaccine), ratio estimate for geometric mean titre (test vaccine divided by licensed vaccine).

Table 2: **Seroconversion (by PRNT50 antibody assay) and geometric mean titre on day 56 in per-protocol population (n=735)**

analysed during the assay validation phase against a panel of JEV isolates. Results indicated that seroconversion was highly correlated between all isolates tested. Geometric mean titres varied between strains, with SA_{14}-14-2 giving representative titres that closely matched results to wildtype isolates.

Statistical analysis

The primary objective of the study was to show the non-inferiority of the test vaccine versus the licensed vaccine in terms of seroconversion rates and geometric mean titres at day 56. Non-inferiority of the test vaccine would be shown if the lower limit of the 95% CI for the seroconversion difference (ie, test vaccine minus licensed vaccine) was greater than –10% and if the lower limit of the 95% CI for the geometric mean titre ratio (ie, test vaccine divided by licensed vaccine) was greater than 1–1·5. We calculated that 792 participants (396 per group) would be needed to show non-inferiority of the seroconversion rate with a two-sided 95% CI and power of 80%, assuming a seroconversion rate of 75% in both groups and an SD (log_{10} units) of 0·4 for the geometric mean titre.

Primary immunogenicity assessments were based on the per-protocol population (n=735), excluding participants with major protocol violations or who were seropositive for Japanese encephalitis at baseline. We assessed non-inferiority of the coprimary endpoints with two-sided 95% CIs. A Mantel-Haenszel-type statistic stratified for study site and age group (<50 years *vs* ≥50 years) was used for the seroconversion rate difference. A PROBIT analysis was done to calculate the $PRNT_{50}$ titre from plaque counts for different dilution factors for every participant and visit. The resulting $PRNT_{50}$ was thereafter log-transformed to compare the geometric mean titre ratio (or difference of log-transformed values) between vaccination groups. A fixed effect was assumed for the study site as well as age and treatment group in this ANOVA.

We used an analysis of variance (ANOVA) for study site, age group, and treatment group to the log_{10}-transformed $PRNT_{50}$ values; the resulting treatment difference and confidence limits were retransformed to the original scale to obtain the geometric mean titre ratio with confidence limits. All secondary immunogenicity data were based on the intention-to-treat population (n=867). The safety analysis population (n=863) included participants who entered into the study and received at least one vaccination.

This trial is registered as a clinical trial, EudraCT number 2004-002474-36.

Role of the funding source

The study sponsor planned and coordinated the study, did the PRNT analyses, and drafted the final clinical study report for regulatory purposes. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

With respect to baseline demographics of the study participants, those at US study sites were slightly older than their European counterparts (table 1). On day 56 (28 days after the second vaccination for the test vaccine and 28 days after the third vaccination for the licensed vaccine), we recorded 98% seroconversion in participants who received the test vaccine, compared with 95% in those who received the licensed vaccine (95% CI for the difference –1·33 to 3·43). We recorded a geometric mean titre of 244 for the test vaccine group compared with 102 for the licensed vaccine (table 2). Based on the frequency of $PRNT_{50}$ titres on day 56, the test vaccine group showed a substantially higher geometric mean titre than did the licensed vaccine group (figure 2). Seroconversion and geometric mean titre data did not show any significant effect of age on the immune response to the test vaccine.

Overall, the local tolerability and general safety profile of the test vaccine was promising in the study, with no

serious safety concerns recorded. The most common adverse events after immunisation were: headache (113 [26%], test vaccine; 125 [29%], licensed vaccine), myalgia (88 [21%]; 69 [16%]), influenza-like illness (54 [13%]; 55 [13%]), and fatigue (54 [13%]; 48 [11%]). Frequency of adverse events was similar between treatment groups, and vaccine-related adverse events were generally mild. One serious adverse event was reported: a 50-year-old man developed a myocardial infarction 3 weeks after the last vaccination with the test vaccine. This event was regarded as probably not related to the vaccine itself. The participant was then treated with four coronary stents after which the adverse event was resolved.

The most common local side-effects were hardening, swelling, and redness at the injection site, with substantially higher reporting rates in the licensed vaccine group. Figure 3 presents severe symptoms within 1 week after every vaccination: 1% (n=4) of participants in the test vaccine group reported redness compared with 11% (46) in the licensed vaccine group (0·7% [n=3] *vs* 5·3% [n=23] for swelling; 1·0% [n=4] *vs* 5·2% [n=25] for hardening). This difference was more pronounced after the third and second injections than after the first. The difference for severe events was also greater than that for moderate events, which in turn was greater than that for mild events. Participants reported similar frequencies of local tolerability symptoms after injections of the test vaccine on days 0 and 28 compared with placebo on day 7. Additionally, we recorded no changes in haematology or clinical chemistry laboratory results in either study group during the study.

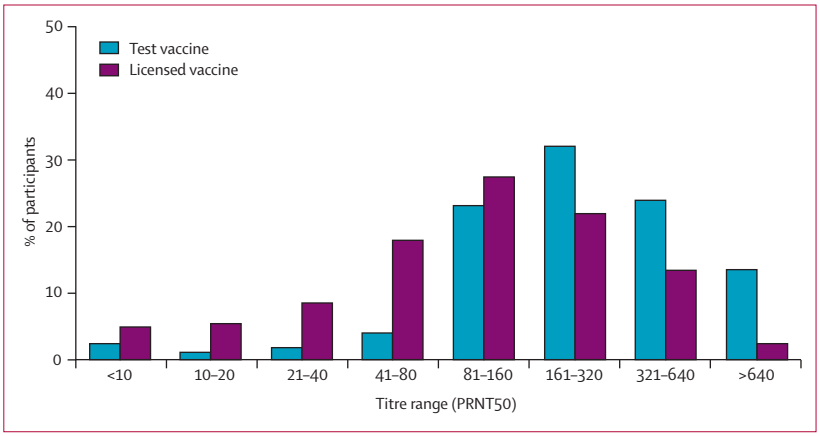


Figure 2: **Frequency distribution of JEV-neutralising antibody levels at day 56 in immunised participants**
Number of participants based on per-protocol analysis (test vaccine, n=361; licensed vaccine, n=364). PRNT50=50% plaque reduction neutralisation test.

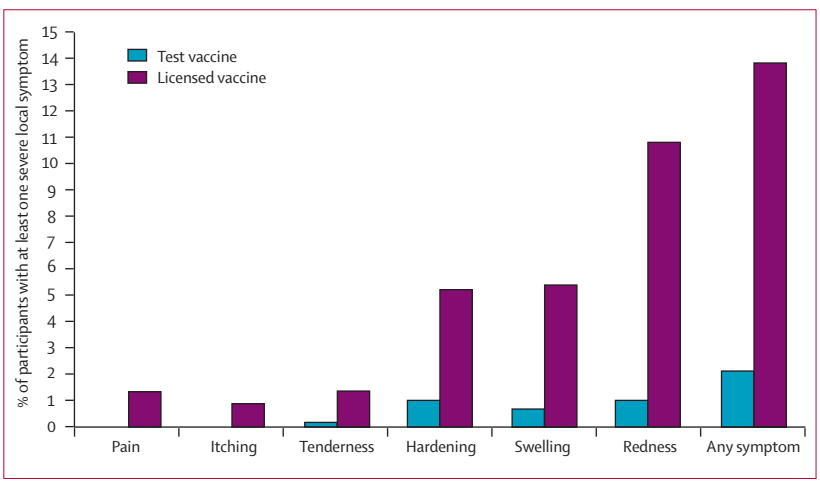


Figure 3: **Participants with at least one severe local symptom 0–6 days after vaccination (on days 0 or 28)**
Fisher's exact test for any symptom (9/421 [2%] *vs* 59/427 [14%]): p<0·0001.

Discussion

Our direct comparison with the currently licensed vaccine has shown that the Intercell test JEV vaccine is at least equivalent with respect to immunogenicity and antibody titres. Since the clinical efficacy of the licensed vaccine has been shown previously, the non-inferiority results of the test vaccine with respect to serological variables suggest at least an equal clinical efficacy.

JEV infections are regarded as one of the most serious viral causes of encephalitis, with a mortality of up to 30–50% and a high percentage of neurological sequelae in survivors.[2] Thus, mass immunisation programmes against Japanese encephalitis are generally recommended for populations residing in the endemic areas by regional and international public-health authorities, including WHO.

In developed, non-endemic countries, Japanese encephalitis is regarded as a rare and exotic disease. But in recent decades, case reports of infections in tourists and other travellers from non-endemic regions have been reported almost every year.[10,15,16] However, vaccine coverage in the population of international travellers at risk is very low, which is not only due to a lack of awareness of the disease on the part of travellers and their travel health advisers, but also because of fear of the potential adverse reactions associated with the currently licensed mouse-brain-derived JEV vaccine.[17]

Mouse-brain-derived JEV vaccines have been widely used in various countries in Asia and in some developed countries for decades. In adults immunised in Australia, Europe, and North America, serious adverse reactions have been reported, consisting of urticaria or angio-oedema and, in some cases, dyspnoea. The occurrence of these adverse reactions varies and ranges from less than 1 to 104 per 100 000 injections, with anaphylaxis as one of the major causes for concern.[6,18–21] Moreover, recent cases of disseminated encephalitis in Japanese children have been suggested to be related to vaccination with mouse-brain-derived JEV vaccine, which led to a suspension of the recommendation to use this vaccine type in the Japanese infant immunisation programme[16] and a cessation of vaccine production by

major Japanese manufacturers. Therefore, use of this vaccine type will be restricted to few regions in the future. A live, attenuated vaccine also using the strain SA_{14}-14-2 was already used in China, Nepal, Sri Lanka, and Korea, and has now also been used in India since 2006.[22] Potential safety hazards with live vaccines, especially with those from genetically modified strains, are a constant source of controversy in the scientific community and among public-health officials,[4,23,24] with the potential threat of mutagenic reversion into pathogenic strains as the greatest fear among critics. Systemic adverse events after immunisation, such as fever and headache, were similar in frequency, as seen in a Chinese study using a live vaccine derived from the same strain as the test vaccine in our study.[25]

Despite the potential safety risks associated with current vaccines, both inactivated and live-attenuated, these vaccines have been proven to be lifesaving and are still an important measure to fight Japanese encephalitis in many countries. Although a field study to prove the efficacy of a new JEV vaccine would be desirable, it is not feasible.[26,27] Since the test vaccine was compared with a licensed product that had been shown to be protective under field conditions, and since positive PRNT titres after the licensed vaccine could be correlated to protection, JEV-neutralising antibody titres (measured by PRNT) could be used as a surrogate marker for protection in recipients of the test vaccine.

The correlation of PRNT titres with protection has been shown in previous non-clinical and clinical studies, and has been widely discussed and accepted in platforms such as WHO.[7,12,27] A virus-neutralising antibody threshold of 1:10 dilution in a 50% PRNT assay is regarded as protective.[8,12,26,28] However, this assumption did not hold true for measles in a outbreak in 1985.[29] The present study showed that 98% of participants receiving the test vaccine who completed the protocol had JEV-neutralising antibody titres above the protective threshold of a titre of 1:10 with a geometric mean titre of 244.

Notably, the immunogenicity results were obtained with only two doses of the test vaccine 4 weeks apart, whereas three doses of the licensed vaccine were needed to achieve similar results. The protective efficacy obtained with only two injections will make the test vaccine more convenient to use in mass vaccine programmes, and more convenient to schedule for international travellers. Additionally, fewer vaccine doses will also reduce potential side-effects.

Safety and tolerability with the test vaccine were excellent in this study. However, we cannot provide evidence for the absence of very rare systemic side-effects described after immunisation with the conventional mouse-brain-derived JEV vaccine. Nevertheless, the test vaccine will probably not have the same potential for serious adverse reactions, since neurological adverse events after use of mouse-brain-derived vaccines are attributable to impurities from mouse-brain-derived proteins.[28,30] Although described with cell-culture-derived smallpox vaccines, so-called innocent bystander effects, which are triggered by microglial activation leading to myocarditis in rare cases, are very unlikely for an inactivated vaccine such as the test vaccine used in our study.[31,32]

A safe and effective JEV vaccine is needed to protect residents of endemic regions, as well as others at risk (eg, travellers and military personnel). With the increasing numbers of world travellers visiting rural Asia where Japanese encephalitis is endemic, the growing risk of transmission to travellers will increase the need for pretravel immunisation with a safe, convenient, and immunogenic vaccine product. Further investigation of the vaccine might include large-scale surveillance studies to detect rare adverse events, such as delayed hypersensitivity that is seen with the licensed vaccine.[33] Furthermore, efforts will also be needed to supply this vaccine to children living in endemic countries. First studies in India are currently in preparation with a locally produced vaccine using the identical manufacturing process and technology. As with all modern vaccine studies, international consensus on vaccine safety surveillance studies is needed (eg, the Brighton Collaboration).[22] Additional studies are also underway to analyse cell-mediated immune responses induced by Japanese encephalitis vaccination. This new, second-generation, Vero-cell-derived JEV vaccine, containing the purified, inactivated JEV strain SA_{14}-14-2 adjuvanted with aluminium hydroxide, seems to be a promising candidate.

Contributors

Intercell AG coordinated the study, did all the PRNT analyses, drafted the final clinical study report for regulatory purposes. The study was planned by Intercell AG and external consultants, some of whom (BJ, HK, and EJ) also served as study investigators in the study. Monitoring, data management, and statistical analyses were done by an independent contract research organisation (Parexel, Berlin, Germany).

Conflict of interest statement

ET, SD, and CSK are employees of Intercell AG. All other institutions received funding for contract research in conjunction with this clinical trial, from which salaries of investigators have been partly covered.

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