Bioterrorism & Biodefense

McArthur and Holbrook, J Bioterr Biodef 2011, S1
http://dx.doi.org/10.4172/2157-2526.S1-002

Review Article Open Access

Japanese Encephalitis Vaccines

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Abstract

Japanese encephalitis (JE) is a significant human health concern in Asia, Indonesia and parts of Australia with more than 3 billion people potentially at risk of infection with Japanese encephalitis virus (JEV), the causative agent of JE. Given the risk to human health and the theoretical potential for JEV use as a bioweapon, the development of safe and effective vaccines to prevent JEV infection is vital for preserving human health. The development of vaccines for JE began in the 1940s with formalin-inactivated mouse brain-derived vaccines. These vaccines have been shown to induce a protective immune response and to be very effective. Mouse brain-derived vaccines were still in use until May 2011 when the last lots of the BIKEN® JE-VAX® expired. Development of modern JE vaccines utilizes cell culture-derived viruses and improvements in manufacturing processes as well as removal of potential allergens or toxins have significantly improved vaccine safety. China has developed a live-attenuated vaccine that has proven to induce protective immunity following a single inoculation. In addition, a chimeric vaccine virus incorporating the prM and E structural proteins derived from the live-attenuated JE vaccine into the live-attenuated yellow fever 17D vaccine virus backbone is currently in clinical trials. In this article, we provide a summary of JE vaccine development and on-going clinical trials. We also discuss the potential risk of JEV as a bioweapon with a focus on virus sustainability if used as a weapon.

Keywords: Japanese encephalitis; Flavivirus; Arbovirus; Vaccine; Biothreat; Biodefense

Introduction

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus (Family Flaviviridae, Genus Flavivirus) endemic to Eastern and Southern Asia and Indonesia and has been isolated in Northern parts of Australia. A disease similar to Japanese encephalitis (JE) was first described in the late 1800s, but the first clearly identified epidemic occurred in Japan in 1924 with a second large epidemic in 1935 [1]. These were followed by regular outbreaks in Japan from 1946-1952 [2]. The last significant outbreak of JE in Japan occurred in 1968. JE was first reported in Korea in 1949, China in 1940, Nepal in 1978 and in a number of other Asian countries since the 1950s [3]. JE was first identified in India in 1954 and has subsequently become a significant health concern in India with an estimated 7500 cases per year and morbidity rate up to 1.5 cases per 100,000 population [3]. In 1995 JEV was identified in a human case in the Torres Strait region of Australia [4] with a subsequent incursion into the Cape York area of mainland Australia in 1998 [5]. JE exists in two distinct epidemiological zones. JE is considered endemic in most tropical regions of Asia and Indonesia with cases occurring year round, but with large outbreaks occurring during the rainy season when mosquito populations increase. In temperate regions of Asia, JE occurs only in epidemics or outbreaks during the warm summer months when mosquitoes are abundant.

JEV is a member of the JE serocomplex of flaviviruses which also includes West Nile virus, Murray Valley encephalitis virus and St. Louis encephalitis virus among others. The first isolate of JEV (type strain Nakayama) was made in Tokyo in 1934 from the brain of a fatal human case [3,6]. Subsequent isolation of the virus in China occurred in 1949 with the isolation of the P3 and Beijing-1 strains from mosquitoes and a fatal human case, respectively [7]. Early immunological characterization of the Beijing-1 and Nakayama strains separated the viruses into two different immunotypes [8]. Subsequent monoclonal antibody analysis identified at least five different antigenic subtypes of JEV that had been circulating since the isolation of the Nakayama strain in 1935 [9-12]. Subsequent genetic analysis determined that the JE subgroup consists of 5 genotypes of viruses [13]. The 5th genotype consists of only a single isolate, the Muar strain which was isolated in 1952 from the brain of a fatal human case from Muar, Malaysia [14,15]. Four of five JEV genotypes (genotypes 1-4) have been isolated in Indonesia while genotype 3 is historically, the most widespread of the 5 genotypes [13]. In the 1990's a shift towards a predominance of genotype 1 seems to have occurred [16,17].

JEV has been isolated from, and can be transmitted by, a number of mosquito species including multiple Culex and Aedes species. Viruses within the JE serocomplex, however, are typically transmitted by Cx. spp. mosquitoes while other mosquito-borne flaviviruses (i.e. dengue and yellow fever viruses) are typically transmitted by Ae. spp. mosquitoes. The principle vector for JEV in Asia is Cx. tritaeniorrhynchus, although members of the Cx. vishnui group have also been associated with the transmission of JEV [3]. JEV can be maintained in mosquito populations by transovarial and trans-stadial transmission and the virus can survive over wintering in dormant mosquitoes [3].

The principal natural reservoirs for JEV include birds and pigs. Many bird species can be infected with JEV, but very few develop disease, indicating that birds may be significant contributors to viral maintenance in nature. Large water birds, particularly herons and egrets, have been suggested to play a role in viral distribution by

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Received July 16, 2010; **Accepted** September 07, 2011; **Published** September 25, 2011

Citation: McArthur MA, Holbrook MR (2011) Japanese Encephalitis Vaccines. J Bioterr Biodef S1:002. doi:10.4172/2157-2526.S1-002

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moving the virus long distances along migratory flyways [3,18]. The expansion of the cattle egret range, in particular, has been suggested to coincide with the spread of JEV [13].

Domestic swine are considered an amplifying host for JEV as they can develop very high viral titers without manifestations of disease. Once a pig is infected, mosquitoes can feed on the pig, become infected and transmit the virus to other pigs, birds, humans or other vertebrates. In many regions of the JEV endemic area, pigs live in relatively close proximity to humans allowing for efficient transmission of the virus from pig populations into the human population. Pigs do not generally show signs of clinical disease although studies in Japan indicate that JEV causes abortion in pregnant sows [19]. Due to the role of pigs in the transmission of JEV, culling of the pig population is a frequent response to JEV outbreaks. Cattle and horses are generally considered dead end hosts for JEV infection as they do not develop sufficient viremia for effective transmission of the virus to biting mosquitoes [20]. Cattle do seroconvert following infection and there is also a suggestion that JEV may be associated with abortion in pregnant cows [21]. Infection of horses can be more severe as they have been shown to develop neurological disease. Studies in the 1960s found a rate of clinical disease of 0.3 per 100,000 horses with a case fatality rate of 42% [22]. The incidence rate decreased to 0.03 per 100,000 with widespread vaccination of horses.

Approximately 3 billion people are thought to be at risk for JEV infection in a region where 20,000 cases and 6000 deaths are reported annually and estimates of up to 50,000 cases per year have been suggested [23]. A significant proportion of susceptible individuals are children as JE is generally considered a childhood disease in endemic regions [23]. In countries where JEV is endemic (i.e. those at warmer latitudes), cases occur throughout the year although an increase in cases is generally noted with increased mosquito abundance in the wet seasons. At cooler latitudes JEV generally occurs in epidemics or outbreaks in the warmer months when mosquitoes are prevalent.

JEV infection can be asymptomatic, develop into a febrile syndrome with headache, or progress to meningitis and/or encephalitis. Severe encephalitis cases initially present as a non-descript febrile illness with severe headache accompanied by dizziness, nausea, vomiting and diarrhea [24]. Additional signs of neurologic disease may include photophobia, altered consciousness, mask-like facies, muscular rigidity, and evidence of tremors or seizures, particularly in children. Death usually occurs between 5 and 9 days following the onset of symptoms. The approximate case fatality rate is around 30% [25]. Many survivors of JE have cognitive and/or physical sequelae including upper and lower motor neuron impairments, deformities in the arms, legs, and feet, language impairment and seizures [25]. There is also evidence of chronic or persistent infections, but this is uncommon [26].

JEV is a single-stranded positive-sense RNA virus with a single open reading frame encoding a single polyprotein. The polyprotein is subsequently cleaved into three structural (capsid (C), membrane (M) and envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Flanking the viral protein coding region are 5'- and 3' untranslated regions. The structural proteins M and E are the principal proteins of the viral particle wherein the E protein is the primary viral antigen and also serves as a receptor binding and fusion protein. The structure for the flavivirus E protein was first solved in the mid-1990s for tick-borne encephalitis virus [27] and subsequently for dengue virus [28]. Additional cryo-electron microscopy analyses of intact pre- and post-fusogenic flavivirus particles have served to more clearly define the flavivirus attachment and fusion process [29]. The viral M protein, and immature version prM, serves to protect the E protein during virus assembly to prevent inadvertent fusion with the host membrane during exocytosis [29]. Proper assembly and release of flavivirus particles requires co-expression of the prM and E genes from an individual virus, which is important for understanding development of novel vaccine strategies for flaviviruses.

Therapeutics

A number of therapeutic strategies have been tested for the treatment of JE. These include compounds tested in rodents or humans with limited efficacy [30-36]. Two drugs which are licensed in the United States, pentoxifylline and mycophenolic acid (MPA), have been shown to have some protective effects when tested against JEV challenge in juvenile mice [30,31]. MPA is an immunosuppressant that is typically used in transplant patients so its use in humans is not practical as it could potentially increase susceptibility to disease or secondary infection. Pentoxifylline (Pentopak™, Pentoxil™, Trental®) is licensed for use in reducing blood viscosity in the case of vascular occlusions or poor circulation in the extremities. Typical off-label uses include treatment of impotence, ulcers and stroke. A clinical study has shown a reduction in the inflammatory response during treatment of patients with chronic hepatitis C [37] yet pentoxifylline was shown to be ineffective in the treatment of SARS coronavirus infection in mice [38]. As with MPA, the effect on human physiology and questionable efficacy in animal models raises significant doubts as to the utility of pentoxifylline in the treatment of JE.

Minocycline, a semi-synthetic derivative of tetracycline, has also been shown to protect mice following challenge with JEV with initiation of treatment 24 hours post infection [39]. It was initially shown in the early 1990s that minocycline was effective against retrovirus infection [40] and more recently to reduce West Nile virus propagation in cell culture [41]. While the specific mechanism of action for treating JEV is unclear, there is increasing evidence that minocycline reduces blood-brain barrier damage by limiting production of reactive oxygen species and induction of apoptosis [42,43] and has also been associated with inducing neuronal repair [44]. The reported effectiveness of minocycline and known tolerability in humans suggests that this agent should be tested in clinical trials for the treatment of JE.

Arctigenin, a lignin derived from the Greater Burdock (*Arctium lappa*), has also been shown to be effective for post-challenge treatment of JEV infection in the juvenile mouse model [45]. Interestingly, arctigenin was also shown to decrease caspase-3 activity (and hence, apoptosis) and to reduce reactive oxygen species by blocking JEV-induced down-regulation of superoxide dismutase production and iNOS up-regulation. Subsequent studies have shown that arctigenin can protect mice from challenge with influenza A [46]. The similarity in activities between minocycline and arctigenin is interesting as both clearly point to the importance of controlling apoptosis and reactive oxygen species in the JEV infected brain.

In clinical studies, the use of interferon alpha-2 in two patients initially looked promising [47], but a subsequent randomized, double-blinded, placebo controlled trial found that interferon alpha-2 was not an effective treatment for JE [48]. Similarly, a controlled trial for the use of ribavirin by oral administration for the treatment of JE found that ribavirin had no effect in reducing mortality associated with JE [49].

Currently, there are no available licensed therapeutic options

for the treatment of JE and supportive care remains the primary treatment option. The identification of potential therapeutic options, particularly minocycline, is encouraging and increases the optimism for identification of appropriate treatment options. One of the biggest challenges for all of the drugs mentioned above is their effectiveness in the clinical setting once symptomatic patients arrive in hospital. In the case of a known exposure (e.g. laboratory setting or intentional/accidental release), the early prevention of viral replication and/or neurological damage may be effective. However, once patients begin showing the neurological signs of disease that are common in JE cases, therapeutic options may be even more limited.

Considerations for biodefense

JEV is considered a potential biothreat agent not only due to potential threats to human health, but also because of agricultural concerns as JEV can affect pig, cattle and horse populations. There is evidence that both the former Soviet Union and Japan evaluated the use of JEV as a bioweapon. However, it is not clear whether or not the virus was ever successfully weaponized or if it would be successful as a bioweapon. Direct aerosol challenge under laboratory controlled conditions was shown to produce lethal infection in squirrel monkeys, hamsters and mice [50]. However, it is not clear that JEV could be successfully delivered by aerosol or that its stablity in the environment would make it an effective weapon. There is also no evidence that JEV can be naturally transmitted by human-to-human contact which would significantly hinder its utility as a weapon against human populations.

The effective use of JEV as a weapon immediately brings to mind the idea of initiating a widespread outbreak of acute disease with significant morbidity. In the case of JEV, establishing an outbreak that progresses beyond the initial point of infection would require appropriate ecological factors to be present. The potential for JEV maintenance in an environment requires consideration for the presence of a competent mosquito vector and potential amplifying hosts. The principal mosquito vector in Asia, Cx. tritaeniorhynchus, is not found in North America, but related species Cx. pipiens, Cx. quinquefasciatus and Cx. tarsalis are found in North America and are considered competent vectors for JEV [51,52]. Aedes spp. mosquitoes, including Ae. aegypti and Ae. albopictus, which are both present in North America, have also been shown to transmit JEV, but are much less efficient than Cx. species [3]. The example of the introduction of West Nile virus (WNV) into the United States in 1999 demonstrated that, while St. Louis encephalitis virus (SLEV) is endemic in parts of North America, additional viruses of the JE serocomplex could be introduced, disseminated and sustained in this ecosystem.

The requirement of amplifying hosts for maintenance of JEV in North America may potentially limit its spread. In Asia, JEV is typically a rural disease that is frequently found in concert with water birds and pigs in an environment where people live in close proximity with these species. In Asia, domestic pigs are a significant component of the JEV transmission cycle and are well known to be amplifying hosts for JEV as they develop a significantly high viremia for effective transmission to mosquitoes [53]. In North America, domestic livestock are not typically held in large numbers in close proximity to human population centers which should reduce the likelihood of explosive outbreaks in human populations. However, the possibility of JEV being introduced into and maintained in large piggeries should be considered a real threat to the farming industry even though the potential impact on human health is limited. The potential for local JE outbreaks near small farms or piggeries remains a risk if the virus is introduced and competent vectors are present in sufficient numbers to facilitate efficient transmission of the virus.

Large water birds such as herons and egrets play an important role in the transmission cycle of JEV as they can develop sufficiently high viremia to allow infection of a biting mosquito and their wide migratory range can allow dissemination of the virus [54-56]. Multiple species of herons and egrets are found throughout North America and could provide a means of virus dissemination if JEV were introduced.

While the introduction of JEV into North America by either natural or intentional means could have a significant immediate health and agricultural impact, the ability of the virus to be maintained is questionable. However, it should also be noted that the ability of WNV to be effectively maintained in North America was questioned and now this virus is endemic. The relative isolation of large piggeries to rural areas should reduce efficient maintenance and transmission of the virus. However, the introduction of JEV into North America (or other non-endemic region) could have a significant economic impact on the pork industry. Culling is often an initial response to JE outbreaks and the presence of JEV in porcine populations could have a negative impact on the export of swine or pork products.

Vaccine development

With the discovery of the causative agent for JE came the first efforts to develop vaccines to prevent infection and to limit the expansion of outbreaks. Initial efforts utilized mice for vaccine development as this technology was known to be effective. With the development of cell culture techniques vaccine production moved into more controlled and predictable vaccine platforms. In recent years vaccine development has begun to utilize recombinant technologies to develop protein subunit vaccines and chimeric virus vaccines. The latter effort utilizes the non-structural protein backbone from the yellow fever virus (YFV) vaccine 17D with the JEV prM and E structural proteins integrated into the genome. This novel vaccine has shown promise and is currently in clinical trials (Table 1).

The incidence of adverse reactions following vaccination has decreased with development of improved manufacturing techniques. These techniques include the limited use of primary animal growth substrates, reduction in preservative use (e.g. thimerisol), improved adjuvants, improved vaccine purity and Good Manufacturing Practices (GMP). In addition, in order to improve data interpretation in clinical trials, recommendations were made to the World Health Organization (WHO) to define the correlate of immunity for JEV vaccines as a 50% plaque reduction neutralization test ($PRNT_{50}$) value of ≥ 1:10 [57]. This value is generally accepted as demonstration of a protective response for all flavivirus vaccines. In addition, demonstration of vaccine efficacy should be made using a licensed JEV vaccine as a direct comparator for a determination of non-inferiority [57].

Formalin-inactivated mouse brain vaccines

Development of first generation JE vaccines began shortly after the discovery of JEV as the causative agent for JE. A collaborative effort between the Rockefeller Institute and the US Army resulted in the development of an inactivated mouse brain-derived vaccine in the 1940s with further development of a chick embryo based vaccine [58]. In Japan, the first vaccine, a formalin-inactivated infected mouse brain homogenate based on the Nakayama strain of JEV, has been in use since 1954 [59,60]. However, the National Standard for development of JE vaccines has been modified over the course of

time to: 1) Reduce or remove brain material in the vaccines to avoid potential neurologic complications and 2) Improve the purity of the vaccine by ultracentrifugation and protein precipitation [60]. In addition, the strain used for vaccine production in Japan was changed in 1989 from the type strain Nakayama to Beijing-1 as the latter virus had a higher yield during vaccine production and was thought to be more efficacious against a wider range of JEV strains [60]. However, a study directly comparing the vaccines derived from the Nakayama and Beijing 1 strains did not identify significant difference in efficacy between the two vaccines [61]. The Nakayama strain was still used for vaccine development in several Asian countries including Korea, Vietnam and India while Beijing-1 was used in Japan and Thailand [59]. The inactivated mouse-brain vaccine was marketed as either BIKEN® or JE-VAX® by the Research Foundation for Microbial Diseases of Osaka University ("BIKEN®") and distributed by Sanofi-Pasteur until February 2011. The production and distribution of this vaccine has ceased, and the last lots of the vaccine expired in May 2011 [62].

Inactivated cell culture vaccines

The development of the SA14-14-2 live attenuated vaccine (see below) appears to have provided a strong candidate for a very effective single dose vaccine with few vaccine-related adverse events. However, there is resistance in some circles to the use of live-attenuated vaccines due to concerns regarding viral reversion to virulence and the use of live vaccines in potentially immunocompromised individuals. Furthermore, inactivated mouse brain-derived vaccine could potentially contain contaminating biological material that could cause allergic reaction or disease. Subsequently, the development of inactivated cell culture vaccines for JEV has been a principal focus. The use of cell culture systems provides several advantages over the use of infected mouse brain for vaccine production. First is cost, as

Vaccine	Manufacturer	Vaccine Type	JEV Strain	Dose Schedule	Licensure	Vaccinee Ages	Notes
JE-VAX®	BIKEN	Inact.-MB	Nakayama Beijing-1	2 doses at 0 and 28 days; boost 3 yrs	Worldwide	≥ 1 yr	JE-VAX is no longer manufactured; existing stocks expired in May 2011
JEBIK V	BIKEN	Inact.-Culture	Beijing-1	2 doses at 6-26 day interval; boost at 1 yr	Japan	≥ 6 months	Currently suspended from pediatric vaccination schedule in Japan.
KD-287	Kaketsuken	Inact.-Culture	Beijing-1	3 dose regimen	Japan	≥ 6 months	Also known as ENCEVAC®, JEIMMUGEN INJ
SA-14-14-2	Chengdu Institute	Live-attenuated	SA14-14-2	Single dose; boost at 9 mo. or 1 yr	China, Nepal, Korea, India	≥ 9 months	Also known as CD.JEVAX®
IC51	Intercell AG	Inact.-Culture	SA14-14-2	2 doses at 0 and 28 days; boost 1 yr	United States, Europe, Canada, Australia, Hong Kong and Switzerland	≥ 17 yr	Also known as IXIARO® and JESPECT®; long-term efficacy undetermined
JE-CV	Saofi-Aventis	Live-Chimeric	SA14-14-2	N/A	In Clinical trials	N/A	Also called IMOJEV or ChimeriVax™-JE; prM/E of JE-SA14-14-2 cloned into YFV-17D backbone; high seroconversion rate following 1 or 2 doses

Table 1:

Clinical Trial	Vaccine	Sponsor	Location	Principal Objective	Start Date	End Date	Projected Enrollees	Notes
NCT01047839	IC51	Intercell AG	USA, Australia, Denmark, Germany, Sweden	Determine safety and immunogenicity in children	Jan. 2010	Nov. 2011	100	Vaccine also marketed as IXIARO® or JESPECT®
NCT01092507	JE-CV	Sanofi-Aventis	Thailand	Demonstrate non-inferiority of one dose of JE-CV compared to one dose of live-attenuated SA14-14-2	Mar. 2010	Oct. 2015	300	Includes long-term follow-up studies out to 5 years post vaccination
NCT01158599	IC51	Intercell AG	Austria, Germany	Determine safety and immunogenicity in the elderly	Jun-10	Jun-11	200	Vaccine also marketed as IXIARO® or JESPECT®
NCT01190228	JE-CV	Sanofi-Aventis	Philippines	Determine safety and immunogenicity of one dose of JE-CV; evaluate severe adverse events for 5 years	Aug. 2010	May-16	505	Immunogenicity also followed to 5 years post-vaccination
NCT01188343	JE-CV	Sanofi-Aventis	Taiwan	Compare immunogenicity of JE-CV alone or when given in combination with measles-mumps-rubella vaccine	Aug. 2010	Jan. 2012	550	Separate and co-immunization of JE-CV and MMR; children 12-18 mo.
NCT01150942	KD-287	Boryung Pharma.	Korea	Assess safety and immunogenicity of inactiveted JE vaccine in children aged 12-23 mo.	Aug. 2010	Aug. 2012	188	Three dose vaccine regimen
NCT01335412	IC51	Intercell AG	USA-Virginia	Surveillance for serious adverse events in active military personnel vaccinated with XIARO	Mar. 2011	N/A	20,000	Electronic surveillance program for all vaccinated military personnel; no enrollment

Table 2:

the generation of virus in cell culture is much more cost effective than using mice. Second, appropriate cell culture systems, such as Vero cells, are devoid of neurological components which caused concerns with the inactivated mouse brain vaccine. Third, quality control is easier in cell culture vaccines as components of the vaccine can be carefully regulated to avoid the presence of serum, antibiotics or other potential immunogens. Fourth, viruses with established immunogenicity and efficacy can be used for development of vaccines.

In 1968 China initiated use of the P3 strain of JEV in cell culture based vaccines generated in PHK (primary hamster kidney) cells [63-65]. The inactivated P3 cell-culture based vaccine was then moved to a Vero (non-human primate kidney) cell culture system. The Vero cell-based vaccine was licensed in 1998 and is now the principal inactivated JE vaccine currently used in China [63-65].

Two Vero cell-based vaccines are currently in use. In Japan, an inactivated vaccine using the Beijing-1 strain is currently in use. This vaccine, JEBIK V (produced by BIKEN), induced higher neutralization indices in mouse trials than did the inactivated mouse brain-derived vaccine at equivalent doses [66]. JEBIK V has been in use in Japan since 2009. The KD-287 (JEIMMUGEN INJ.) (produced by Kaketsuken) vaccine is also based on the Beijing-1 strain of JEV. This vaccine has been licensed for use in Japan and is currently undergoing additional clinical trials in children in Korea.

An inactivated cell culture-based vaccine using the attenuated SA14-14-2 strain has been licensed for use in many countries as they transition from the inactivated mouse brain JE-VAX vaccine, the final doses of which expired in May 2011. The new vaccine, developed by Intercell and known as IC51 or IXIARO® (JESPECT® in Australia), is approved for use as an adult vaccine (for persons ≥ 17 years of age) in the United States, Europe, Canada, Australia, Hong Kong and Switzerland. The vaccine is currently undergoing a number of clinical trials to determine safety and efficacy in children. Clinical trials in adults have shown similar immunogenicity and tolerability when compared to JE-VAX [67-72]. The IXIARO® vaccine has also been shown to be compatible with previous vaccination against tick-borne encephalitis virus [73] and co-vaccination against hepatitis A virus [72]. Current dosing recommendations in the United States indicate a two dose initial series administered 28 days apart for persons ≥ 17 years of age. The booster schedule has not been determined empirically, but published studies indicate the presence of a protective antibody titer 12 months after the initial dose regimen [67].

In the United States, the IXIARO® vaccine is not licensed for use in people < 17 years of age. Until May 2011 the final lot of JE-VAX was being reserved for use in minors, but those lots have expired. Subsequently, there is currently not a licensed pediatric vaccine JEV vaccine available in the United States. A pediatric clinical trial using the IXIARO® vaccine was recently completed in India and additional studies are on-going in the Philippines and in the United States. JEV vaccines, specifically SA14-14-2 are available for pediatric use in Asia.

Updates on on-going clinical trials for numerous therapeutics and vaccines can be found at: www.biocanary.com or www.clinicaltrials.gov (Table 2).

Safety and efficacy of inactivated vaccines

The inactivated mouse brain vaccines have proven to be safe and efficacious over the course of nearly 60 years of clinical use. The efficacy of the mouse brain-derived vaccine has been shown indirectly by decreases in the number of JE cases in areas of significant vaccine coverage [59]. Additionally, two specific clinical trials in children evaluating vaccine protection, one in Taiwan and the other in Thailand, have shown an 80-90% vaccine protective efficacy following a two dose vaccine regimen [74,75].

Improvements in the content and purity of the mouse brain-derived vaccine over the course of time have improved the safety of the vaccine and reduced concerns regarding allergic reactions to the vaccine and adverse neurological events. Reported side effects following vaccination include localized tenderness and swelling in approximately 20% of vaccinees [63-65] with about 10% of vaccinated children in Japan showing similar reactions [59] while more severe complications such as headache, myalgia and fever occur in ~10% of all vaccinees [63-65]. The occurrence of severe neurological complications, including acute disseminated encephalomyelitis (ADEM) occurs at a rate of 1-2 per 100,000 vaccinations given in Japan [59] with apparently higher frequency in Denmark and lower frequency in the United States [76, 77]. The occurrence of ADEM is not exclusive of JE vaccinations as it has also been associated with a number of vaccines including those for rabies, measles, mumps, smallpox, influenza and hepatitis B and is typically associated with primary vaccination [78-80]. ADEM can also occur as a post-infectious complication following many types of viral and bacterial infections [81].

Inactivated cell culture-derived vaccines have also been shown to be safe and efficacious in humans. The PHK cell-derived vaccine used in China has shown up to 95% protective efficacy in trials in China. Over 70 million doses of the PHK vaccine were distributed annually in China prior to the licensure of the live-attenuated vaccine in 2006 [64].

The IXIARO® Vero cell-based vaccine has also been shown to be safe in a number of clinical trials. Adverse events seen in these studies are similar to those seen following vaccination with the mouse brain-derived vaccine, including pain and tenderness at the vaccine site [69,82]. Although direct protection studies have not been reported, vaccination with the IXIARO® vaccine has been shown to produce a strong protective (i.e. neutralizing) antibody response in several studies. The use of a two-dose primary vaccination regimen was shown to improve the protective antibody response with 113 of 115 vaccinees (97.3%) demonstrating a protective antibody response at day 56 post immunization [83]. Neutralizing antibody titers remained at protective levels in 151 of 181 vaccinees (83%) 12 months post-vaccination [67]. As mentioned above, on-going clinical trials should provide substantially more data regarding the quality of the IXIARO® vaccine over the next several years.

Live-attenuated vaccine

In the 1970s Chinese scientists began development of a live-attenuated JE vaccine based on the SA14-14-2 strain. The SA14-14-2 vaccine is currently produced in PHK cells by the Chegdu Institute of Biological Products and was licensed in China in 1988 with more than 300 million doses produced since licensure. The SA14-14-2 vaccine has also been licensed in Nepal, Korea and Sri Lanka with additional licensure currently being sought. The SA14-14-2 vaccine is a component of childhood immunization programs in both Nepal and China [63]. Unlike the inactivated virus vaccines, the SA14-14-2 vaccine requires only a single dose to induce a protective response [84]. The SA-14-14-2 vaccine is the vaccine of choice in China over the inactivated PHK cell-based vaccine.

Safety and efficacy of the live-attenuated vaccine

Since its development, more than 300 million doses of the live attenuated SA14-14-2 vaccine have been produced in China with more than 120 million doses administered to children [63,64]. The SA14-14-2 vaccine has proven to be quite effective with up to 95% efficacy following a single inoculation and up to 96% efficacy as evaluated 5 years following a single dose [84]. Additional studies indicated a 97.5% efficacy following a boost at 1 year after the initial inoculation [85]. The recommended dosing strategy for the SA14-14-2 vaccine is a two dose schedule one week apart with a recommended boost at 9-12 months following initial vaccination.

Several safety studies performed in China have shown no significant indication of adverse events following vaccination [86,87] and no indication of viral reversion to a neurovirulent phenotype.

Chimeric vaccines

In 1999 scientists at OraVax described the production of ChimeriVax-JE, a novel vaccine that incorporated the structural proteins prM and E from the live-attenuated JEV vaccine strain SA14-14-2 into the non-structural protein backbone of the live-attenuated YFV vaccine strain 17D [88]. ChimeriVax-JE was shown to be both immunogenic and protective in small scale non-human primate studies following a stringent intracerebral (IC) challenge [88] and to have reduced neurovirulence following IC challenge in suckling mice [89]. Subsequent analyses provided efficacy data in primates and also led to development of additional vaccine platforms for related flaviviruses, dengue virus and West Nile virus. In initial clinical trials, ChimeriVax-JE vaccination was shown to induce short-term viremia, but was well tolerated and induced production of protective antibodies [90]. Subsequent analyses found that ChimeriVax-JE was protective against the four principal JEV genotypes (1-4) in a mouse model [91]. In addition, mosquitoes fed artificial blood meals containing high titers of ChimeriVax-JE did not become infected with the virus indicating that mosquitoes are unlikely to transmit the virus from a vaccinated individual to another host [92]. ChimeriVax-JE, now known as JE-CV or IMOJEV® and manufactured by Acambis/Sanofi-Aventis has proven safe, immunogenic and effective in a number of human trials [93,94]. Most of these trials involved adult volunteers, but several ongoing phase II and phase III clinical trials involve both adults and children > 9 months of age (www.clinicaltrials.gov).

Summary

While JE is a significant health concern for local populations and travelers in parts of Asia and Indonesia, its effectiveness as a bioweapon is limited. The inability of JEV to be easily transmitted human-to-human by aerosol limits the utility of JEV as a weapon. The use of safe and effective vaccines and a probable inability to be easily maintained in nature would limit the impact of the virus in a non-native habitat. The JEV vaccines currently in use or in development have been shown to be safe and effective in clinical trials. Although the use of the novel IXIARO® vaccine in children is limited in some countries due to a lack of clinical safety data, the proven efficacy of inactivated vaccines in this population should support emergency use in an outbreak.

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