Japanese encephalitis: pathogenesis, prophylactics and therapeutics

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Japanese encephalitis (JE) is one of the most dreaded mosquito-borne viral encephalitis known to afflict humans. The Japanese encephalitis virus (JEV) is a neurotropic flavivirus that affects the CNS, causing extensive damage that may lead to fatality in about one third of patients. Half of the survivors suffer from severe neuropshychiatric sequelae. With nearly 3 billion people living under the current JE-endemic region, recurring incidents of epidemic are being reported at regular intervals. With no established antiviral therapies against JE available, vaccination has been the only way of preventing JE. Two types of JE vaccines are currently in vogue although the safety of administering them is questionable, in certain individuals. Thus, there is a need to develop a safe, affordable and potent JE vaccine and this review addresses the current efforts in this direction. This review also focuses on the pathophysiology of JE and efforts towards a possible breakthrough in anti-JEV therapy.

Keywords: Central neurons system, flavivirus, Japanese encephalitis virus, prophylaxis, vaccine,

Origin and spread of JEV

FROM the 1870s, recurring epidemics of encephalitis have been reported from the islands of Japan, especially during the summer season, with major outbreaks occurring every 10 years. This summer encephalitis was termed as type B encephalitis, to differentiate from von Economo's encephalitis lethargica. The name type B was later dropped and in 1935 the Nakayama strain of Japanese encephalitis virus (JEV) was isolated from the brain of a fatal case[1]. The virus was later classified as a member of the genus Flavivirus (family *Flaviviridae*) named after the prototype Yellow fever virus (Latin; *flavi* = yellow). The genus consists of over 70 other closely related virus species. The origins of JEV are uncertain, but phylogenetic comparisons with other flaviviruses suggest that it evolved from an African ancestral virus, perhaps as recently as a few centuries ago[2] and evolved into its present form in the Indonesia–Malaysia region[3]. Since then,

the virus has spread in South Asia, Southeast Asia, East Asia and the Pacific regions[4]. Rapid globalization, population explosion, changes in global climatic condition, industrialization and deforestation, all seem to correlate with the spread of the virus into newer territories. Today, with approximately three billion people living in the JE-endemic region, there are estimated 35,000–50,000 cases and 10,000–15,000 deaths annually, thereby making JE one of the most dreaded vector-borne viral encephalitis in the world[5] (Figure 1).

In India, JE as a disease was first reported in 1955 when clinical cases were detected in Vellore and Pondicherry in southern India. The virus was however, not recovered from humans until 1958, when three isolations were made from the brain tissue of infected persons. Until early 1970s, the disease was reported only from southern India with periodic focal reports of its occurrence. A major outbreak resulting in 42.6% case-fatality rate was reported from Bankura district of West Bengal in 1973. Subsequently, the disease spread to other states and caused a series of outbreaks in different parts of the country. In 1978, cases were reported from 21 states and union territories, and from then onwards till 2007 there have been 103,389 reported cases of JE in India that has led to 33,729 deaths[6]. Approximately 597,542,000 people in India live under the JE-endemic regions and there are 1500–4000 reported cases every year[7]. These figures are based on total reported cases and it is quite possible that several cases may go unreported and hence the actual magnitude of the threat of JE may be considerably higher both in the Indian as well as the global context.

Enzootic cycle of JEV

JE is a vector-borne viral disease. JEV is transmitted between vertebrate hosts by mosquitoes belonging to the *Culex* sp. (Figure 2). The virus is able to replicate within the salivary glands of the mosquitoes. Mature JE virions remain entrapped in intracellular vacuoles and are later released into the apical cavity of salivary gland cells through the fusion of these vacuoles with the apical plasma membrane. This process is associated with primary re-synthesis of saliva in mosquitoes following blood feeding activity. Another type of shedding involves
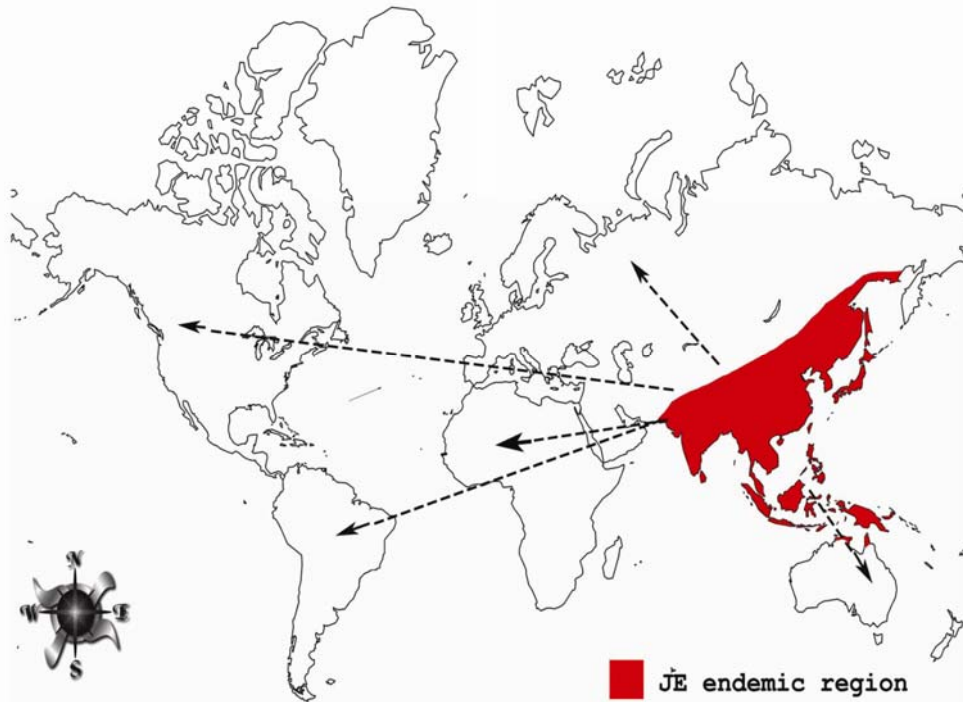
Figure 1. Japanese encephalitis (JE) endemic regions. The current JE-endemic region consisting of entire Southeast Asia, parts of Russia and Australia. The possible direction of spread of the disease is shown as broken arrows. (Figure not to scale; adapted from Ghosh and Basu, *PLoS Neglected Tropical Diseases*, 2009.)
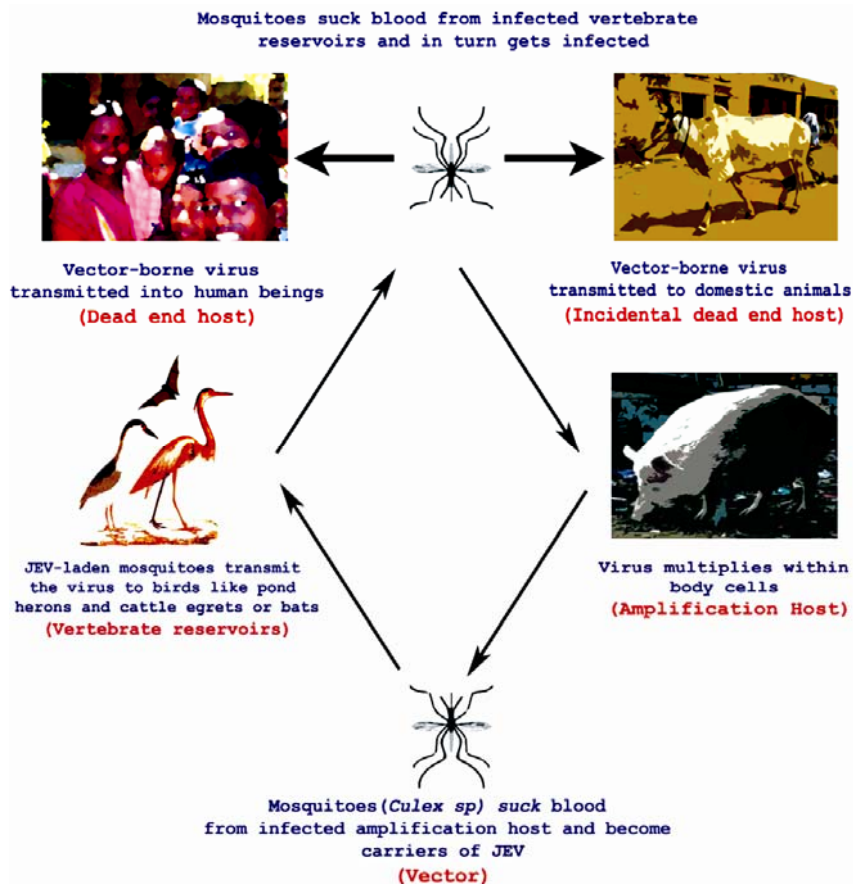

Figure 2. Enzootic transmission cycle of JE virus. The virus is transmitted to vertebrate hosts by mosquitoes belonging to the *Culex* sp. Pigs serve as amplification hosts and forms a critical link in the transmission cycle. Ardeid water birds and bats serve as virus reservoirs. Humans are dead end hosts as the virus cannot be transmitted from an infected person to another.

virus particles, either singly or in mass, being released directly through the apical plasma membrane[8]. Components of the mosquito saliva may also modulate infection by altering the local cytokine milieu. Feeding by mosquitoes of *Culex* sp. or administration of sialokinin-I, a mosquito salivary protein, have been found to downregulate IFN-γ production and upregulate the TH$_2$ cytokines, IL-4 and IL-10 (ref. 9).

Human infections are mainly spread by *Culex tritaeniorrhynchus* which breeds in pools of stagnant water such as rice paddy fields[10]. Because the rice paddy is unavoidable, majority of the population in rural Asia has been infected with the virus by early adulthood[11]. Wading ardeid water birds (e.g. herons and pond egrets) and bats serve as virus reservoirs or maintenance hosts, but the virus regularly spills over into pigs, members of the family of Equidae (e.g. horses and donkeys) and humans. Pigs are considered as the main amplifications hosts as viraemia results with a high titre. Due to the close proximity of pigs with human dwellings these animals are considered main component in the transmission cycle with respect to human infection[12]. JEV infection in other domestic animals does not result in high viraemia and thus, they are not expected to transmit the virus to humans.

Molecular architecture of JEV

The JE virion consists of a single strand of positive-sense RNA of around 11 kilobase, inside a nucleocapsid and is surrounded by a glycoprotein-containing envelope. The RNA comprises a short 5′ untranslated region (UTR), a longer 3′UTR and a single open reading frame between them. It codes for a single polyprotein, which is translationally and post-translationally cleaved by viral and host proteases into three structural proteins (core-C, premembrane-PrM and envelope-E), and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The C protein (12–14 kDa) is highly basic and combines with the RNA to form the nucleocapsid[13]. The prM is closely associated with the E protein, forming a heterodimer and is thought to act as a 'chaperone' to it, impairing its function until after virion release. Immediately prior to virion release, the prM protein (18–19 kDa) is cleaved to its mature M protein (8–9 kDa) form. This allows the formation of E protein homodimers, which are thus 'activated'. The prM protein of JEV contains a single N-linked glycosylation site, which is highly conserved among the JEV strains. Researches indicated that this highly conserved N glycosylation motif in prM is crucial for multiple stages of JEV biology: prM biogenesis, virus release and pathogenesis[14]. The E protein is the largest structural protein (53–55 kDa), with up to two potential gylcosylation sites. It is the major target for the humoral immune response and is thought to be important for viral entry into host cells. It is worth mentioning that low pH is extremely important for viral entry into the cell to trigger

viral membrane fusion with host endosomal membrane, thereby releasing the nucleoplasmid in the cytosol.

Pathophysiology and clinical manifestations of JE

The course of JEV infection, starting from its entry till reaching its site of action, the central nervous system (CNS), is not well-defined. Studies with other flaviviruses have led to the belief that upon entry through mosquito bite, the virus infects Langerhan's dendritic cells in the skin[15] and is carried to nearest draining lymph nodes[16], thereby initiating a round of early immune response. Unfortunately for the host, this is not sufficient to counter the virus and it spreads to secondary lymphoid organs before entering the blood circulation through the efferent lymphatic system. During the ensuing transient viraemia, peripheral organs such as kidney, liver and spleen are known to be infected[17], after which the neurotropic flavivirus spreads to the CNS. How JEV manages to evade the host's peripheral immune response is another matter of speculation, as there are no definitive studies regarding this aspect, till date. Once the virus evades the immune system, it crosses the blood–brain barrier (BBB) to enter the CNS. JEV may cross the BBB by passive transport across the endothelium, by active replication in endothelial cells, or by a 'Trojan horse' mechanism in which the virus is carried into the brain by infected inflammatory cells[18]. Monocytes and macrophages are thought to be probable carriers of the virus into the CNS as studies have shown that the virus can survive for a prolonged time and effectively replicate within these cells[19,20]. The structural and functional integrity of the BBB is critically compromised during JE[21] which may possibly be caused by matrix metalloproteases released in the infected brain[22]. Due to the impaired functioning of the BBB, peripheral inflammatory cells are recruited to the infected brain at later stages of JE that exacerbates the damage.

JEV causes extensive neuronal damage in the brain, though in many cases, the virus is probably not directly involved in the destruction of brain tissue but may cause damage indirectly by triggering cell-mediated immune response by activating microglia. Microglias are the resident immune cells of the CNS and have a critical role in host defense against invading microorganisms. Microglial activation is viewed as an adaptive response whereby microglia release neuroprotective factors to facilitate the recovery of injured neurons and they also phagocytose dying or damaged neurons, before they lyse and release toxic agents into surrounding areas. JEV infection has been shown to activate microglia both morphologically and functionally, *in vivo*, that leads to an elevation of proinflammatory mediators, such as IL-6, TNF-α, RANTES and MCP-1. These proinflammatory mediators and cytotoxins released from activated microglia are instrumental in inducing neuronal death that accompanies JE[23].

Neuronal death by secreted TNF is mediated by the TNF receptor-associated death domain protein (TRADD)[24], which there upon regulates a downstream apoptotic cascade in neurons. However, neuronal death also activates microglia and astrocytes and thus, the inflammatory cycle goes on. Nitric oxide (NO) also plays an important role in inflammation during JE infection, although NO itself is a strong antimicrobial agent researchers have shown that it profoundly inhibits viral RNA synthesis, viral protein accumulation and virus release from infected cells[25]. Thus, NO may play a crucial role in the innate immunity of the host and its ability to restrict the initial stages of JEV infection in the CNS.

Besides neuronal and microgial cells, studies have shown that astrocytes are also infected by JEV[26]. Astrocytes are known to maintain homeostasis in the CNS and to support the survival and information processing functions of neurons. They respond promptly to CNS infection and help regulate neuroinflammation[27]. JEV infection results in astrocyte activation, but the infection overwhelms the capacity of even activated astrocytes to maintain metabolic homeostasis, resulting in an over accumulation of toxic byproducts of metabolism that are detrimental to neuronal viability. Though JEV infection triggers metabolic reprogramming by upregulating the expression of several proteins such as IP-10 (ref. 28), ceruloplasmin and glutamine synthetase, involved in the metabolic pathways crucial for maintaining neuronal health, this increase is insufficient to meet the increased demand that accompanies JEV infection[29]. Astrocytes, being a component of the BBB, may also help in the transmission of JEV from peripheral tissues to the cerebrospinal fluid. JEV also causes the upregulation of certain host genes in the brain. An *in vivo* study using subtraction hybridization technique has identified several genes whose expression is upregulated by JEV in mouse brain[30,31]. It has been further demonstrated that several of these genes are also induced in the CNS by rabies virus and Sindbis virus. Thus, it appears that different neurotropic viruses activate common host cell pathways during infection of CNS[30,31].

JE typically develops in patients after an incubation period of 5–15 days[32]. In humans, most JEV infections are asymptomatic, with about one in 300 JEV infections resulting in symptoms ranging from non-specific febrile illness to severe meningoencephalitis, characterized by fever, reduced consciousness, seizures and focal neurological signs[33]. At later stages, poliomyelitis-like flaccid paralysis and parkinsonian syndrome develops, which manifests the classic description of JE – dull, flat, mask-like face with wide, unblinking eyes, tremor, generalized hypertonia, cogwheel rigidity, and other abnormalities in movement[34]. Histopathological and imaging studies have shown JEV infection and inflammation in the anterior horn cells of the spinal cord, which provided an anatomical correlate for these presentations[35]. Radiological studies also support the earlier observations from pathological

investigations implicating the basal ganglia, especially the substantia nigra and thalamus in the parkinsonian syndromes seen in JE, which include tremor, cogwheel rigidity and mask-like faces[36]. Recently, there has been further molecular evidence of the involvement of nigrostriatal pathway in JE survivors with movement complications. In the study, (99m) Tc-TRODAT-1 and (123)I-IBZM SPECT imaging revealed that presynaptic dopaminergic neurons in JE patients are more susceptible to JEV than post-synaptic striatal neurons[37]. Electroencephalographic abnormalities observed in JE patients include the presence of alpha, theta and delta coma, and epileptiform conditions[38]. Symptoms of brainstem infection include changes in the respiratory pattern, flexor and extensor posturing, and abnormalities in the papillary and occulocephalic reflexes[39]. In fatal cases of JE, pathological changes are polymorphic and diffuse, involving various parts of the nervous system where the brain shows a severe degree of vascular congestion, microglial proliferation and formation of gliomesenchymal nodules, focal or confluent areas of cystic necrosis, cerebral oedema and transcompartmental shift[40]. Many survivors of JE acquire neuropsychiatric sequelae with cognitive and language impairment, in which case the disease presents itself not only as a killer but also as a cause of an immense social and financial burden, especially for a developing country[33]. Recently, it has been reported that neural progenitor cells (NPC) are permissive to JEV infection both *in vivo* and *in vitro*, which leads to their growth retardation. The pathophysiological relevance of these observations was supported by profound decrement in actively proliferating NPCs in the subventricular zone (SVZ) of JEV-infected animals. Infection of the NPCs and suppression of their proliferation might be primarily responsible for dysregulated neurogenesis and development of cognitive deficits in survivors of JE[41].

Prophylactic strategies against JE

Due to lack of definitive therapeutic countermeasures to combat JE, implementation of prophylactic strategies remains the best way to prevent JE. The most important aspect of the prevention of JE spread is vector control. Paddy fields not only provide an ideal breeding ground for mosquitoes but also attract migratory birds, thereby helping the spread of the virus. Responsible use of larvicides, insecticides, and adopting ecofriendly methods such as using neem cakes and growing larvivorous fish are useful in controlling mosquitoes in paddy fields[4]. As pigs are the amplification host in the transmission cycle of the virus, close monitoring of domesticated animals is necessary. Vaccinating pigs is also a logical way to acquire protection against JEV by breaking the mosquito–pig–human transmission cycle[42].

Human vaccination remains, till date, the most effective measure to prevent JE. Multiple vaccines exist to

control JE, but all have limitations. Unfortunately, unlike smallpox, it is difficult to eradicate JEV by vaccination, because human beings are actually a dead-end host of the virus. The formalin-inactivated vaccine against JEV was produced from infected mouse brain-derived tissue soon after the virus was discovered. This type of vaccine, manufactured by the Research Foundation for Microbial Diseases of Osaka University, Japan, became commercially available in Japan (as the Japanese Biken vaccine JE-VAX) and was produced in Korea by the Green Cross Vaccine company. These were later licensed to be produced in the United States also[43]. This is the only JE vaccine recommended by the World Health Organization (WHO), but there have been several concerns with its side effects[44]. These vaccines are expensive and require multiple doses to maintain efficacy and immunity. The Biken vaccine is no longer produced. The Korean Green Cross vaccine needs to be administered also in three doses on day 0, 7 and 28 after birth, with boosters given every 12 months following primary course, then every three years, unless at particular risk in which case annual boosters are recommended. This makes its use difficult for people in developing countries. Researchers have been trying to avoid a multidose requirement of the vaccine by using adjuvants such as biodegradable poly (gamma-glutamic acid) nanoparticles and alum[45]. Another mouse brain inactivated JE vaccine was produced in India by the Central Research Institute (CRI) in Kasauli[46] but the production of the vaccine was suspended from 2007 as the institute had failed to upgrade its main laboratory according to the GMP norms of WHO[47]. An inexpensive, live attenuated vaccine (SA14-14-2)[48] was licensed by China in 1988, but WHO does not approve it for human use because it is produced in primary hamster kidney cells. Initially, this vaccine was used almost exclusively in China and parts of Korea, but it is now being widely used in the Indian subcontinent as well as Nepal. The vaccine seems to be highly effective, and very few adverse effects have been observed following its use in Nepal[49]. No information is, however, available on the efficacy and side effects of this vaccine in India. Neuroattenuation of the virulent JE SA14-14-2 strain is reported to be based on 57 nucleotide changes and 24 amino acid substitutions, suggesting that reversion to neurovirulence of the vaccine strain is unlikely. Though the vaccine was adjudged to be safe and efficacious by the WHO's Global Advisory Committee on Vaccine Safety, several parameters such as safety in immunocompromised individuals and pregnant women, viral shedding in vaccines and implications of the shedding, efficacy in infants less than one year of age, remains to be ascertained[50]. The recently developed Vero cell-derived inactivated JE vaccine containing the purified, inactivated JEV strain SA14-14-2 with aluminum hydroxide as adjuvant seems to be a promising candidate and has passed the phase III randomized controlled trial[51]. Technology for a Vero cell-derived vaccine based

on an Indian strain of JEV[52] has been developed and the vaccine is under pre-clinical development.

Several efforts have been and are still being made to develop recombinant vaccines for JEV. A highly attenuated recombinant vaccinia virus, NYVAC[53] was used for JE vaccine development which proved to be quite successful[54]. Other strains tested include modified vaccinia virus Ankara strain[55,56]. Later studies focused on safe replication-competent recombinant viruses such as avipoxviruses and ALVAC, which infect cells but do not replicate[56,57]. In another research on peptide-based vaccine, a JEV E protein was fused to Johnson grass mosaic virus coat protein by using recombinant DNA technology[58].

Researchers have also constructed the Yellow fever virus to express JEV protein, the most promising recombinant vaccine under development. Widely known as ChimeriVax-JE, this vaccine incorporates the structural proteins (prM and E) of the JEV SA14-14-2 strain into YF17D, the yellow fever vaccine[59–61].

Attempts have been made to generate DNA vaccines for JE and efficacy of various plasmid DNA constructs encoding the JEV E protein or prM has been evaluated in murine and primate models[62–65]. A single intramuscular injection of recombinant plasmid DNA containing cDNA encoding JEV prM and E proteins induced protective immunity and prevented JE in mice and their progeny[63]. Again, investigators have used both intramuscular injection and a gene gun to deliver the plasmid gene for E protein and prM protein[64]. DNA immunization with colloidal gold, construction of chimeric DNA vaccine vectors are the focus of some of the other recent promising studies[65,66]. A novel genetically engineered DNA vaccine against flavivirus has been proposed in which the consensus sequence of structural domain III of the E protein of the virus that is reported to carry dominant epitopes that induce neutralizing antibodies, has been created. This has been administered along with a genetically engineered IL15 DNA vaccine molecular adjuvant for co-stimulating the immune response against DIII clones. This has resulted in a 4–5 fold increased immune response[67]. In another strategy, needle-free jet injection of a mixture of JEV DNA and protein vaccines was shown to enhance the immunogenicity of the DNA vaccine in a murine model[68]. Recently, pseudoinfectious JE viruses (named RepliVAX JE) lacking the JEV capsid protein capable of replicating in normal cells but incapable of producing infectious particles have been shown to protect mice and hamsters from lethal JEV challenge indicating that the RepliVAX platform can be used for producing a safe and potent JE vaccine[69].

Therapeutic measures against JE

Chemotherapy against JE is at present supportive and not targeted at the virus. Interferon therapy has not met with

Table 1. Promising preventive and therapeutic strategies for Japanese encephalitis

Prophylactic strategies – vaccine development

ChimeriVax-JE	Incorporates the structural proteins (prM and E) of the JEV SA14-14-2 strain into YF17D, the Yellow fever vaccine
RepliVAX JE	Pseudoinfectious JE viruses lacking the JEV capsid protein capable of replicating in normal cells but incapable of producing infectious particles
Vero cell-derived inactivated JE vaccine	Contain purified, inactivated JEV strain SA14-14-2 with aluminum hydroxide as adjuvant; has passed phase III randomized control trials; Vero cell-derived vaccine based on an Indian strain of JEV has also been developed and is currently under pre-clinical development
Recombinant vaccinia and avipoxvirus	JE vaccines using recombinant strains of vaccinia virus and avipoxvirus have been shown to be quite effective
Peptide-based vaccine	JEV E protein was fused to Johnson grass mosaic virus coat protein by using recombinant DNA technology
DNA vaccines	Efficacy of various plasmid DNA constructs encoding the JEV E protein or prM has been evaluated; DNA immunization with colloidal gold, construction of chimeric DNA vaccine vectors has also been found to be effective; the consensus sequence of structural domain III of the E protein of JEV that is reported to carry dominant epitopes that induce neutralizing antibodies, has been created an administered along with a genetically engineered IL15 DNA vaccine molecular adjuvant for co-stimulating the immune response against DIII clones

Therapeutic strategies – drug development

Rosmarinic acid	Reduces JEV replication in mice brain and also ameliorates the secondary inflammation resulting from microglial activation
Arctigenin	Confer substantial protection in a murine model from GP-78 strain of JEV infection by markedly decreasing virus-induced neuronal apoptosis, microglial activation, active caspase activity and induction of proinflammatory mediators in the brains
Curcumin	Dysregulates ubiquitin-proteasome pathway to inhibit infective viral particle formation in *in vitro* studies; reduces JEV-induced cellular stress
N-methylisatin-β-thiosemicarbazone derivative	Inhibits JEV replication
Glucosidase inhibitors	Block the trimming step of N-linked glycosylation, thereby eliminate the production of several endoplasmic reticulum-budding viruses, including JEV
Minocycline	Reduces neuronal apoptosis, microglial activation, active caspase activity, proinflammatory mediators and viral titer; possess antioxidative properties that significantly ameliorate the oxidative stress generated as a result of JEV infection; imparts protection to the blood–brain barrier that is severely compromised in JE
siRNA	Single intracranial administration of lentivirus-delivered short hairpin RNA or lipid-complexed siRNA before viral challenge or siRNA treatment after viral challenge has been found to be sufficient to provide protection against lethal encephalitis

great success[70]. Over-expression of interferon stimulating gene 15 has been found to impart protection against several viral infections[71–74] and may also work against JEV. A recent clinical trial with oral administration of ribavarin, an antiviral drug that interferes with RNA metabolism required for viral replication, was also not encouraging[75]. A naturally occurring compound discovered to have antiflaviviral activity and the potential to be developed as an antiflaviviral drug is rosmarinic acid (RA). RA, a phenolic compound found in various Labiatae herbs, possesses several anti-inflammatory and antioxidative properties. An *in-vivo* study conducted with GP78 strain of JEV in murine model has showed that RA reduces viral replication in mice brain and also ameliorates the secondary inflammation resulting from microglial activation[76]. Arctigenin, a naturally occurring phenylpropanoid dibenzylbutyrolactone lignan is known to possess antioxidant, anti-inflammatory, neuroprotective and antiviral properties. It has been reported that treatment with arctigenin confer substantial protection in a murine model from GP-78 strain of JEV infection by markedly decreasing virus-induced neuronal apoptosis, microglial activation,

active caspase activity and induction of proinflammatory mediators in the brains of arctigenin-treated mice[77]. The study also claims that arctigenin may act directly on brain cells, because neuronal cell line was also salvaged from JEV-induced cell death. Curcumin, a naturally occurring phenolic compound extracted from *Curcuma longa* L., has been shown to impart neuroprotection against JEV infection in an *in-vitro* study. Curcumin possibly acts by decreasing cellular reactive oxygen species level, restoration of cellular membrane integrity, decreasing pro-apoptotic signalling molecules, and modulating cellular levels of stress-related proteins. It has also been shown that the production of infective viral particles from previously infected neuroblastoma cells is reduced, which is achieved by the inhibition of ubiquitin-proteasome system[78].

A remarkable achievement in anti-flaviviral drug research is the discovery of antiviral effect of minocycline, a member of the broad-spectrum antibiotic tetracycline group[79]. A significant piece of research on minocycline as an anti-JEV drug is an *in-vivo* study[80] that showed minocycline reduces neuronal apoptosis, microglial activation, active caspase factivity, proinflammatory mediators and

viral titer markedly on the 9th day post infection. Mino-cycline was also shown to possess antioxidative properties that significantly ameliorate the oxidative stress generated as a result of JEV infection[81]. Minocycline also imparts protection to the BBB that is severely compromised in JE[22]. Another compound that has shown inhibition of JEV replication completely *in vitro* is an *N*-methylisatin-β-thiosemicarbazone derivative[82].

Glucosidase inhibitors of the endoplasmic reticulum, such as *N*-nonyl-deoxynojirimycin, which block the trimming step of *N*-linked glycosylation, have been shown to eliminate the production of several endoplasmic reticulum-budding viruses, including dengue type II (DEN-2) and JEV[83]. In a novel approach, a synthetic oligonucleotide-based DNAzyme targeting the 3'NCR of JEV was shown to significantly inhibit virus replication in cultured cells and mouse brain and protected the JEV-infected mice from death[66]. In yet another innovative study on mice using RNA interference, a single intracranial administration of lentivirus-delivered short hairpin RNA or lipid-complexed small interfering RNA (siRNA) before viral challenge or siRNA treatment after viral challenge was sufficient to provide protection against lethal encephalitis. Interestingly, when a cross-species conserved sequence of cd-loop coding viral envelope protein was targeted, encephalitis following both JEV and WNV challenge was avoided. This result clearly indicates that by careful drug design of the conserved target site, a single siRNA treatment could suppress viral infection across species, thereby augmenting the treatment of acute viral infections with overlapping clinical symptoms[84].

Future perspectives

With the advent of modern technologies and advancement of knowledge, research in drug and vaccine development against JE has been going on at a steady rate for the past two decades. Though a lot of progress has been made in unraveling the mysteries of the virus that has helped in planning and designing countermeasure strategies, the real success would be when an effective drug or vaccine would be easily available to the masses exposed to the threat of JE, at an affordable cost. Table 1 summarizes the most promising prophylactic and therapeutic approaches against JE. It has been hypothesized that this virus may spread to greater geographic areas apart from its current endemic region. Thus it would be prudent to be prepared for epidemics of greater magnitudes.

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