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Does antiviral therapy have a role in the control of Japanese encephalitis?

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Abstract

Approximately 2 billion people live in countries where Japanese encephalitis (JE) presents a significant risk to humans and animals, particularly in China and India, with at least 700 million potentially susceptible children. The combined effects of climate change, altered bird migratory patterns, increasing movement of humans, animals and goods, increasing deforestation and development of irrigation projects will inevitably lead to further geographic dispersal of the virus and an enhanced threat. Although most human infections are mild or asymptomatic, some 50% of patients who develop encephalitis suffer permanent neurologic defects, and 25% die. Vaccines have reduced the incidence of JE in some countries. No specific antiviral therapy is currently available. Interferon alpha-2a was tested in a double-blind placebo-controlled trial on children with Japanese encephalitis, but with negative results. There is thus a real need for antivirals that can reduce the toll of death and neurological sequelae resulting from infection with JE virus. Here we briefly review the epidemiological problems presented by this virus, the present state of drug development and the contributory role that antiviral therapy might play in developing future control strategies for JE.

Keywords: Antivirals; Antiviral therapy; Japanese encephalitis; Japanese encephalitis virus; Control strategies

1. Introduction

Japanese encephalitis virus (JEV) is the major mosquito-borne encephalitic flavivirus pathogen in south-east and eastern Asia, where it has been responsible for over 50,000 cases of encephalitis annually with a fatality rate in symptomatic cases of between 20 and 30% (Umenai et al., 1985; Vaughn and Hoke, 1992; Endy and Nisalak, 2002; Solomon et al., 2003a,b). Animal immunization and/or vector control policies in endemic countries are impractical; human vaccination is therefore considered the most effective long-term control measure (Hoke et al., 1992; Igarashi, 2002; Tsai, 2000). Although extensive vaccination has virtually eliminated JE in some countries, during the past decade the disease has spread to Pakistan (Igarashi et al., 1994) western India (Prasad et al., 1993) (Dhanda et al., 1997), New Guinea (Johansen et al., 2000) and the Torres Strait of northern Australia (Hanna et al., 1996) (Mackenzie, 1997; Hanna et al., 1999; Mackenzie et al., 2002b), with the prospect of further spread (Mackenzie et al., 2007).

Unlike poliovirus, for which humans are the only hosts, JEV is enzootic, and therefore will never be eradicated from the natural environment. Thus, even though vaccination can reduce its incidence, effective antiviral therapy could supplement existing strategies for controlling this disease. To date, there is only one report of a placebo-controlled clinical trial in which sufficient numbers of recognized JE cases in children were identified and treated with interferon alpha-2a, known to be effective against JEV under laboratory test conditions (Solomon et al., 2003a). Unfortunately, the outcome at discharge and 3 months did not differ between the treated and placebo group.

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In this article we briefly review basic information on the virus and the epidemiology of JE, describe current vaccines and control strategies, then discuss experience with the experimental therapy of JEV infection *in vitro* and in animal models and examine how an effective drug might be advanced to human clinical trials.

2. Classification

JEV is a member of the genus *Flavivirus* in the family *Flaviviridae*, which contains many closely related human pathogens, including yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), Murray Valley encephalitis virus (MVEV), St Louis encephalitis virus (SLEV), tick-borne encephalitis virus (TBEV), Omsk haemorrhagic fever virus (OHFV) and Kyasanur Forest disease virus (KFDV). The family *Flaviviridae* also contains the genus *Hepacivirus*, hepatitis C virus (HCV) (Thiel et al., 2005).

Five distinct genotypes of JEV have been described (Uchil and Satchidanandam, 2001; Solomon et al., 2003b) but only four have been rigorously confirmed (Gould et al., 2004; Mackenzie et al., 2007). Recombination has been described between these genotypes (Twiddy and Holmes, 2003; Gould et al., 2004), complicating the problem of disease control. Genotypic variation may be of concern for vaccine development, since it frequently involves the E protein and selection of escape mutants that evade pre-existing immunity, and could theoretically impact on the response of the genotypes to therapeutic treatments. However, there is little evidence of significant variation in the enzymatic domains among the JE genotypes, and this probably bodes well for antiviral therapy.

3. Replication strategy

JEV is an enveloped virus with a single-stranded, positive-sense, 10–11 kb RNA genome. The 5′ untranslated region (UTR) is capped and variable in length (Gritsun and Gould, 2007b). The 3′ end is not polyadenylated (Burke and Monath, 2001; Lindenbach and Rice, 2003) and is polymorphic (Hanna et al., 1996; Poidinger et al., 1996; Nam et al., 2002; Gritsun and Gould, 2007a). The genome contains a single open reading frame (ORF) encoding a polyprotein that is processed by co- and post-translational cleavage. The virus structural proteins, capsid (C), envelope (E) and membrane (M), which is derived by post-translational cleavage of the PrM protein, are situated towards the 5′ end of the genome.

The non-structural (NS) proteins, NS1, NS2A, NS2B, NS3, NS4A, NSS4B and NS5 provide the replicative, morphogenetic and polyprotein processing functions of the virus during infection and replication (Rice et al., 1985; Lindenbach and Rice, 2003). These enzymes are distinct from equivalent cellular enzymes and are potential targets for antivirals. A reasonable strategy for developing antiviral therapy against JEV would therefore be to identify a drug that targets another medically important flavivirus, such as HCV, although conservation between the enzymatic domains of these two viruses is relatively low (de Lamballerie et al., 2002). Nevertheless, virus helicases have common tertiary structure, despite their limited amino acid homology (Lin and Kim, 1999). Thus, comparative tests on flaviviruses using antivirals effective against HCV enzyme sites are justified.

4. Epidemiology

Pigs, migrating birds, and ornithophilic mosquitoes, especially *Culex tritraeniorynchus* (Mitamura et al., 1938), play an important role in the amplification, dispersal and epidemiology of JEV (Buescher and Scherer, 1959; Buescher et al., 1959a,b; Vaughn and Hoke, 1992). Humans and horses are only incidental hosts for the virus. In endemic areas, most adults are immune to JEV through previous exposure, making it mainly a disease of children and the elderly. However, disease may occur at any age in humans arriving from non-endemic areas, or when outbreaks occur outside endemic areas. The complex ecology makes disease control difficult. In many countries in which JE is a serious problem, the facilities for surveillance, diagnosis, and disease control are often poor.

Two epidemiological patterns are recognized. The disease is either endemic with sporadic cases throughout the year or in temperate/northern tropical regions, it is epidemic with outbreaks in summer or early autumn after the rainy season. The annual disease burden approaches 175,000 cases (Tsai, 2000) although this is probably a significant underestimate. Generally, when the climate favours high mosquito population densities, this coincides with the most significant epidemics. Efforts to introduce new drug therapies should therefore focus on times and places where the disease occurs most frequently. Epidemiologists could contribute by identifying the best sites for drug trials.

5. Clinical syndrome

Amongst indigenous populations, JEV infection causes a variety of clinical manifestations, from non-specific febrile illness to severe meningoencephalomyelitis, with a ratio of symptomatic to asymptomatic infections commonly between 1:50 and 1:300. In contrast, non-indigenous US servicemen showed unusually high rates of 1:25 and 1:63 (Halstead and Grosz, 1962; Benenson et al., 1975). Sub-clinical infections usually result in protective immunity (Mackenzie et al., 2002a; Solomon and Vaughn, 2002).

Encephalitis is often preceded by fever, headache, gastrointestinal symptoms, deteriorating state of consciousness, and neck stiffness. Sudden onset with fever and convulsions may occur in children or occasionally in adults, and is a bad prognostic feature. Cranial nerve palsies are common and patients may demonstrate either flaccid or spastic paralysis. Severe cases progress to coma with respiratory failure requiring ventilatory support. Tremor, cogwheel rigidity, cerebellar ataxia and upper limb weakness are seen occasionally and about 25% die.

Recovery commences after about 1 week, but neurological deficits may take months to resolve. Up to 50% of patients show long-term residua including cranial or peripheral

nerve palsies, epilepsy, blindness, Parkinsonism and movement disorders. In children residual behavioural and psychiatric disturbances are common, including memory impairment, emotional lability and aggressiveness. Neurological disease may also present without overt signs of encephalitis. It is a common cause of polio-like acute flaccid paralysis in children in endemic areas (Solomon et al., 1998), and may present as acute psychosis or as benign aseptic meningitis (Kuwayama et al., 2005). JEV infection in pregnancy is rare, but may result in intrauterine foetal infection and death (Chaturvedi et al., 1980).

6. Pathogenesis

Following infection by mosquito bite, JEV enters dendritic cells under the skin. They transport it to peripheral lymph nodes where virus replication occurs. Following viraemia, usually lasting less than 1 week, most patients begin to recover. Alternatively, the virus enters the central nervous system probably through penetration of the vascular endothelium. Evidence of entry by way of the olfactory nerve has been observed in laboratory animals. Once in the brain, the virus infects neuronal cells and later, phagocytic cells. CNS infection occurs within the hippocampus, thalamus, substantia nigra, and brainstem. The temporal lobes, the cerebellum and the upper spinal cord, particularly the anterior horn cells, may also be involved. These areas show inflammatory cell infiltration and oedema, with a predominance of activated T cells, macrophages and B cells. The inflammatory response is important in causing cerebral disease, although other mechanisms such as apoptosis and viral replication probably contribute to the damage (Mackenzie, 2005).

7. Diagnosis

Because the viraemic period for infected individuals is only a few days, virus isolation from blood and CSF is difficult. Whilst new molecular methods are becoming available antibody detection continues to be the mainstay of diagnosis during the early stages of the disease (Kabilan et al., 2004). Early detection of IgM in the CSF or serum is a reliable indicator of JEV infection. Antigenic cross-reactivity between related flaviviruses, such as DENV and WNV can complicate broadly reactive tests such as ELISA and/or haemagglutination–inhibition.

In addition to serologic testing, accurate diagnosis is aided by consideration of the time of the year, the region in which the case is occurring, an association with paddy fields or pigs, etc., in addition to the clinical findings. Accordingly, in certain geographic areas, a large percentage of children presenting to hospitals with fever and signs of CNS infection have JE. Although rapid diagnosis using RT-PCR might be important for designing a clinical trial of a new antiviral therapy, this would require highly trained personnel and expensive on-site equipment. If an epidemic occurs in a localized region, and large numbers of children require hospital treatment, retrospective confirmatory diagnoses could be equally informative.

8. Treatment

There is no specific therapy for JE. Guidelines for clinical care in JE are available (www.liv.ac.uk/braininfections). Treatment is supportive, consisting initially of assessment of the level of consciousness and transfer to an intensive care unit. Intubation and protection of the airways reduce the risk of aspiration pneumonia and subsequent hypoxia (Solomon et al., 2000). Oxygen should be given and the patient should have the head elevated to reduce raised intracranial pressure. Hydration status must be carefully assessed, and re-hydration provided as appropriate. However, this must be balanced against the risk to the patient. Mannitol and steroids may be given to alleviate raised intracranial pressure, but opinion is divided as to the benefit of these treatments (Hoke et al., 1992) (Nakano et al., 2003). Seizures are common and require treatment (Solomon et al., 2002). Careful nursing care and physiotherapy can alleviate contractures and bedsores. With good supportive care, mortality rates are reduced from approximately 60–10%.

Corticosteroids and anti-inflammatory drugs have been investigated in the treatment of JE. High-dose dexamethasone was tested on acutely infected JE patients in Thailand, but produced no significant beneficial effects (Hoke et al., 1992). In contrast, methylprednisolone, a glucocorticod used to aid nerve cell regeneration, was studied in non-controlled pulse therapy of humans suffering from viral encephalitis and showed beneficial effects, possibly due to its anti-inflammatory activity (Nakano et al., 2003). The steroid dihydroepiandrosterone, which has been tested as a treatment for cancer, diabetes, obesity, ageing and diseases affecting the immune system, reduced the infectious yield and cytopathogenicity of JEV in cell culture (Chang et al., 2005). The analgesic and anti-inflammatory drug sodium salicylate also suppressed JEV propagation in neuronal and non-neuronal cell cultures, probably by decreasing JEV-induced dephosphorylation of extracellular signal-regulated kinase. Clearly, more studies are required to assess the true value of these treatments.

9. Vaccines

Although several vaccines have been produced and used in Asia, few have been accepted for universal application, partly due to licensing issues in the countries where they are most required (Schiøler et al., 2007) and partly because of the costs of their use and their limited availability, problems that are currently being resolved.

A live-attenuated vaccine (SA-14-42) based on the SA-14 strain of JEV was generated by serial passage in primary hamster kidney cells and has been licensed for use in China since 1988. It is currently administered to approximately 20 million children annually in China, with a regime of one dose at age 1 year followed by a second at 2 years and a third at 6 years of age (WHO, 1998; Tsai, 2000; Monath, 2002) (www.path.org/vaccineresources/japanese_encephalitis-vaccine.php). The Republic of Korea subsequently acquired a commercial licence to manufacture this vaccine and it is also now used in Nepal, Sri Lanka and India (PATH, 2006). Despite its widespread use, there have been some reports of systemic

and neurological complications that raised concerns over safety (Nothdurft et al., 1996; Plesner et al., 1996; Takahashi et al., 2000; Shlim and Solomon, 2002; Okabe, 2005). Nevertheless, it now represents approximately 50% of all JE vaccines produced worldwide. Production and control standards continue to be reviewed and further efficacy trials are being carried out (WHO, 2005).

An inactivated mouse brain-derived vaccine, based on the Nakayama or the Beijing-2 strain, was originally developed in Japan, then produced under licence in a number of Asian countries (Barrett, 1997; Tsai, 2000). This vaccine has also been used by many western businessmen and tourists to JE-endemic countries. It had a multi-dose immunization regime with a requirement for a booster every few years; it has recently been replaced by a Vero cell-derived version (PATH, 2006). Similarly, a hamster cell line-derived inactivated vaccine based on the Beijing-3 strain that was developed in China is being replaced by a Vero cell-derived version.

A genetically engineered vaccine based on the YF 17D vaccine strain as a backbone, but with the envelope and premembrane protein genes of YFV replaced by those of JEV virus, has been developed by Acambis (ChimeriVAX^{TM} JE) and tested in clinical trials which are continuing. The results in terms of immunological response and side effects have thus far been very favourable (Acambis, 2007). Whether or not a genetically engineered infectious vaccine will be able to compete with those which are developed by more traditional methods, and are therefore probably cheaper, remains to be seen.

10. Control strategies

The ecology and epidemiology of JE have been extensively reviewed (Endy and Nisalak, 2002). The interplay among rice cultivation, vector densities, and pig rearing close to human habitation is of particular importance for disease control (Mishra et al., 1984; Geevarghese et al., 1994; Gajanana et al., 1997; Kanojia et al., 2003; Phukan et al., 2004). An effective method of preventing the occurrence of JE would be to eradicate all mosquitoes in affected countries—clearly an impossible task! Alternative control strategies have therefore been developed.

In the late 1960s, the incidence of JE was reduced markedly in Japan, Korea, Taiwan and Singapore. Vaccination of humans was probably the major factor, but other activities such as pig and horse vaccination programmes, the widespread use of pesticides in paddy fields, and careful management of pig breeding locations relative to human habitation also contributed to reduction of JE (Okuno, 1978; Igarashi, 1992; Igarashi, 2002). In addition, the disease burden in China has been significantly reduced by vaccination of humans (Halstead and Jacobson, 2003). At the same time, however, there has been an increase in geographic spread and disease incidence in much of southeast and southern Asia, where co-ordinated immunization programmes have not been universally implemented (Lowry et al., 1998; Halstead and Jacobson, 2003; Kabilan et al., 2004). In these areas, disease incidence has probably also been influenced by development of irrigated agriculture and the introduction of new crops.

Major efforts are now being made, particularly in India and Nepal, in a concerted programme of JE disease control, partly funded by the Bill and Melinda Gates Foundation (www.path.org/je), currently based primarily on vaccination of children using a live-attenuated vaccine originally developed in China. However, the potential success of the vaccination campaign in India and Nepal is still only relative. Although there is currently strong support for vaccination, and new, safer vaccines are currently been developed, it will be necessary to sustain a high rate of immunization indefinitely. For example, even if the current vaccination campaign in India and Nepal results in a 95% reduction of cases, hundreds of cases will still arise every year, and the disease burden will remain relatively high.

Other factors are also important in the success of vaccination. The Chinese live-attenuated vaccine, which is the mainstay of the current campaigns, may not always be appropriate. Its efficacy in HIV-positive individuals is not yet clear, and individuals immuno-compromised following steroid treatment, for example, or transplant recipients or pregnant women, may not be suitable for vaccination. Thus, despite the success of the recent vaccine campaigns, JE will not disappear. This is a compelling argument for the development of antiviral therapy.

11. How could antiviral drugs help to control JE?

There are several situations in which effective antivirals might play an important role in health management. In JE-endemic countries with poor health management infrastructures, elderly and malnourished people as well as inadequately or non-immunized children are at greatest risk. Depending on local knowledge of the occurrence of JE cases and the time post-exposure when treatment is initiated, antivirals could reduce the risk to the most vulnerable members of the community by reducing the severity of disease and/or fatality rate in cases admitted to hospital. In epidemic areas where seasonal outbreaks can be reliably predicted, a drug might be made available to children and the elderly, during the epidemic season, as soon as they develop symptoms of JE such as fever and severe headache. However, compared with the lower cost of vaccinating everyone, this would probably not be cost-effective, and would also pose a risk of selecting for drug-resistant viruses.

Tourists and visitors to endemic countries are particularly vulnerable to JE, and health advisers therefore recommend immunization for those visiting endemic regions for 3 weeks or more (Wittesjö et al., 1995). Moreover, the available vaccine for use in western countries is being improved, in terms of purity and reduction of adverse side-effects, but it is still an inactivated virus preparation, requiring multiple doses and a booster every few years. New vaccines such as those based on live-attenuated YF-17D chimeras are also on the horizon and may circumvent problems arising from the use of inactivated vaccines. However, until a live-attenuated, safe, effective and cheap JE vaccine is approved for use outside endemic areas, antivirals that could be made available for treatment of potential cases of JE in visitors might offer a reasonable alternative or adjunct to vaccination, reducing the risk to non-immunized visitors from non-endemic countries.

Antivirals could also be of benefit to workers temporarily employed in areas of high risk of exposure to infected mosquitoes, for example laboratory workers, or businessmen, engineers, etc., entering high-risk geographic regions. By analogy with the closely related WNV, immuno-compromised people in JE-endemic regions, including organ transplant recipients, might also have an elevated risk of encephalitis following exposure to the virus and could benefit from the use of JE antivirals. A possible side-benefit of employing such policies might be that related viruses such as DENV and WNV which overlap geographically with JEV might also be susceptible to the anti-JE drug, and *vice versa*. This would be particularly apt if a medication could be developed that is also effective against HCV, since this would attract major pharmaceutical companies.

12. Experimental development of antivirals against JEV

Since they share similar genome strategies, potential antivirals for individual flaviviruses may display antiviral activity against other viruses within the family (Borowski et al., 2003; Deas et al., 2005). Accordingly, various approaches are being utilised to identify flavivirus chemotherapeutic agents, including, screening known inhibitors of other viruses, rational design based on protein crystal structures or secondary viral RNA structures, optimization of known viral inhibitors, use of humanized antibodies, use of immunoglobulins, and nucleic acid-based therapy (Ray and Shi, 2006). Here we briefly review published reports of experimental inhibitors of flaviviruses, divided into those compounds that block individual steps in viral replication and those, such as interferon, that induce protective host responses.

12.1. Direct inhibitors of viral replication

12.1.1. Inhibitors of virus adsorption and entry

Two types of large molecular compounds, polyoxotungstates and sulphated polysaccharides, impair flavivirus adsorption and entry into host cells *in vitro*, apparently by binding to the cell surface (Shigeta et al., 2003; Talarico et al., 2005). Sulphated galactomannans protected mice from lethal YFV infection when inoculated simultaneously with the virus (Ono et al., 2003).

12.1.2. RNA polymerase inhibitors

The licensed drug ribavirin has been used to treat a number of RNA viral infections. It functions as an RNA cap analogue and mutagen, causing errors in synthetic pathways (Leyssen et al., 2001; Morrey et al., 2002; Leyssen et al., 2005). Ribavirin was effective in the *in vivo* treatment of influenza virus (Gilbert et al., 1985), respiratory syncytial virus (Taber et al., 1983), Lassa fever virus (McCormick et al., 1986) and hantavirus infections (Huggins et al., 1986; Huggins et al., 1991). However, the *in vitro* and *in vivo* activity of ribavirin against YFV and DENV was poor (Huggins et al., 1984; Huggins, 1989; Neyts et al., 1996). In a blinded, placebo-controlled study, prophylactic ribavirin treatment of rhesus monkeys infected with DENV had little effect on viraemia (Malinoski et al., 1990) and in mice, intraperitoneal administration of ribavirin had no effect on survival following intracerebral inoculation with DENV. However, treatment with ribavirin-29,39,59-triacetate, a prodrug of ribavirin, resulted in a significantly increased survival time and rate, possibly due to its higher ability to cross the blood–brain barrier (Koff et al., 1983). Ribavirin also produced beneficial effects on YFV infections in hamsters (Sbrana et al., 2004). The combined use of interferon and ribavirin on humans suffering from chronic HCV infection, produced more promising results than monotherapy with either of the antivirals (McHutchison et al., 1998; Davis, 1999). However, combination therapy in patients dually infected with HCV and hepatitis G virus showed no significant reduction in the HGV levels in serum (Lau et al., 1997; Rostaing et al., 1997; Yang et al., 1998).

Nucleoside analogues, characterized for chemotherapeutic use against HIV and Hepatitis B virus, show inhibitory activity in cell culture against YFV, DENV and WNV (LaColla and Sommadossi, 2004). However, a point mutation in the polymerase active site confers insensitivity to the drugs (Migliaccio et al., 2003; Olsen et al., 2004). Rather than blocking RNA replication, some analogues inhibit flaviviruses by inhibiting nucleoside triphosphate synthesis in host cells. For example, 6-azauridine acetate, pyrazofurin, and 2 thio-azauridine, inhibit orotidine monophosphate decarboxylase (OMPDC). In contrast, mycophenolic acid and ribavirin inhibit inosine monophosphate dehydrogenase (IMPDH) and block viral RNA synthesis (Sintchak et al., 1996; Diamond et al., 2002). Carbamate prodrugs have also been recommended as IMPDH inhibitors since they show *in vivo* activity (Stamos and Bethiel, 2004).

12.1.3. Antisense and siRNA

Nucleic acid-based therapy, using antisense ribozyme and RNA interference-based technologies, have been explored in cell culture to inhibit replication of flaviviruses. Significant reduction of infectivity was observed (Summerton and Weller, 1997; Adelman et al., 2002; McCown et al., 2003; Moulton et al., 2004; Stein et al., 2004; Deas et al., 2005; Kinney et al., 2005). A plasmid designed to express small hairpin RNA against JEV membrane-encoding sequence, injected intraperitoneally immediately following a lethal dose of intraperitoneally administered JEV, protected up to 80% of mice (Murakami et al., 2005). Other *in vitro* and *in vivo* studies showed that phosphorodiamidate-morpholino oligomers were effective in cell cultures infected with JEV, WNV or SLEV and also moderately effective, without toxicity, when inoculated intraperitoneally into mice infected with WNV (Deas et al., 2007). Recently, a conserved 27-nucleotide DNAzyme targeting sequence in the JEV 3′ UTR reduced virus infectivity in mouse brains and extended the survival period of infected animals when administered with a lethal dose of JEV (Appaiahgari and Vrati, 2007).

The use of lentivirus-expressed short hairpin RNA (shRNA) or synthetic short interfering RNA (siRNA) to target either virus-specific or cross-species conserved loop-coding sequences in domain II of the viral envelope protein of JEV or WNV has also produced promising results (Kumar et al., 2006). This target has an essential role in virus-induced membrane fusion. Mice challenged with JEV or WNV were protected by the cor-

responding virus-specific sequence, whereas the cross-species conserved sequence protected mice against both viruses. A single intracranial administration of lentivirus-delivered shRNA or lipid-complexed siRNA protected against lethal encephalitis when administered before or after virus challenge (Kumar et al., 2006).

12.1.4. Inhibitors of other viral enzymes

Flavivirus helicases and proteases are also targets for antivirals, because unlike host cellular proteases and helicases, they recognize cleavage sites containing di-basic amino acids (Frick and Lam, 2006). Compounds that inhibit NS3 protease activity mimic the protease cleavage site, thereby competitively binding to the catalytic site (Nall et al., 2004). Compounds containing single guanidino groups that interact with the S1 pocket in the NS3 protein have also been identified (Chanprapaph et al., 2005).

Some ring-expanded "fat" nucleoside (RENs) and nucleotide analogues that have already been identified as potential antivirals and anti-cancer agents have also been assessed as inhibitors of JEV, WNV and HCV. The most sensitive virus, thus far, appears to be WNV with HCV being the least sensitive (Zhang et al., 2003a,b). RNA aptamers or antibody fragments have also shown potential when tested against HCV and WNV, but considerable developmental work is still required (Frick and Lam, 2006).

12.2. Interferon

The efficacy of interferon-α-2a, -α-2b, -α-1n and of interferon inducers on HCV infection is largely dependent on pre-treatment virus levels, viral genetic diversity, the condition of the liver and immune system of the recipient, and whether or not the patient is immuno-compromised (Davis et al., 1990; Benvegnu et al., 1998; McHutchison et al., 1998; Pawlotsky et al., 1998; Peignoux et al., 1998; Gish, 1999; Gonzalez-Peralta et al., 1999; Lopez Labrador et al., 1999). In the case of flaviviruses, two strains of JEV showed different susceptibilities to inhibition by interferon-α or -β when tested *in vitro* but interferon α-2b was therapeutic when applied to cells infected with WNV (Vithanomsat et al., 1984; Anderson and Rahah, 2002). One case study suggested beneficial effects of recombinant human α-interferon on two patients infected with JEV (Harinasuta et al., 1985). When challenged with lethal YFV, JEV, WNV or SLEV rhesus monkeys, weanling mice or baby hamsters were either protected, or disease severity was reduced following treatment with interferon α-2b or interferon inducers (Stephen et al., 1977; Saxena et al., 2003; Rahal et al., 2004; Sayao et al., 2004; Stamos and Bethiel, 2004). Poorly controlled studies suggested interferon-α-2b reduced the symptoms and duration of disease due to WNV in mice and independently, St. Louis encephalitis virus in humans (Rahal et al., 2004; Sayao et al., 2004). However, a double-blind, placebo-controlled trial in severe cases of JE in children in Viet Nam demonstrated that interferon α-2a treatment produced no therapeutic benefit (Solomon et al., 2003a). It is possible that the differing responses to interferon described above reflect properties associated with flaviviral nonstructural proteins NS4B, NS2A, and NS5, all of which are interferon antagonists (Liu et al., 2004; Best et al., 2005; Muñoz-Jordán et al., 2005).

12.3. Antibodies

Passive transfer of antibodies from humans or mice immune to flaviviruses protected mice challenged intracerebrally with either homologous or heterologous cross-reactive flaviviruses (Gould et al., 1986; Kimura Kuroda and Yasui, 1988; Broom et al., 2000; Ben-Nathan et al., 2003; Ray and Shi, 2006). However, passively transferred antibodies raised against inactivated JE vaccine enhanced the virulence of MVEV in a murine system, but this effect was not observed if the antibodies were raised against live-attenuated JE vaccine (Broom et al., 2000). The reverse was also observed with passively transferred antibodies raised against inactivated MVEV (Lobigs et al., 2003). In some cases, viral virulence was enhanced by the passive transfer of a monoclonal antibody following virus infection (Gould et al., 1987). Nevertheless, TBEV-immune human immunoglobulin, has been passively administered to humans potentially exposed to TBEV in Europe (Kunz et al., 1981), and humanized monoclonal antibody against WNV protected mice following lethal challenge with WNV (Oliphant et al., 2005).

Anecdotal evidence suggests that neutralising JEV monoclonal antibodies tested on JEV-infected humans in China had beneficial effects. Intravenous immunoglobulin containing JEV antibodies has also been used successfully for patients with Japanese encephalitis (Caramello et al., 2006) and West Nile virus encephalitis (Shimoni et al., 2001). A phase I/II trial of intravenous immunoglobulin for WNV infection is being completed by the US Collaborative Antiviral Study Group (http://www.clinicaltrials.gov/ct/show/NCT00069316?order=1).

13. Problems associated with treating CNS infections

Although *in vitro* testing can provide evidence of a compound's potential antiviral properties, with or without associated toxicity, it does not necessarily reflect how the potential drug will perform *in vivo*. Testing in laboratory animals may also give incomplete information to predict clinical efficacy if the antiviral is administered at the same time as the virus challenge, i.e. before infection of the nervous system, since this does not represent the likely situation in the field. It is estimated that CNS involvement in JE probably commences within 4–6 days following infection. Thus, even using the most rapid molecular diagnostic methods, which can detect approximately 65% of cases, there is a risk that the virus will already have invaded the CNS by the time antiviral treatment is initiated. Under such circumstances, many drugs will be clinically ineffective because of their inability to cross the blood–brain barrier.

Of the drugs described above, passively administered antibodies and appropriately delivered inhibitory RNA/DNA molecules appear to show the most promising potential, since some have reduced disease severity in infected animals after it has already progressed to the encephalitic stage. Importantly, the

problem of delivering these RNA molecules intracranially may now have been resolved following the discovery that a potential "carrier" rabies virus glycoprotein peptide efficiently crosses the blood–brain barrier after trans-vascular administration (Kumar et al., 2007).

14. How could antivirals against JE be assessed in clinical trials?

A phase I clinical trial for toxicity could be carried out in any country, and would involve treatment of healthy volunteers. If an antiviral has already passed clinical trials for other purposes, this would simplify the task. Efficacy testing would be facilitated by the fact that JE epidemics occur regularly in southern and southeastern Asia, with children and the elderly typically presenting with the first signs of JE or specifically with encephalitis (Solomon et al., 2003a). Such persons living in an area where an epidemic is known to have commenced could thus be provided with the potential antiviral under trial and subsequently monitored. In India, clinical trials could be performed in hospitals where children frequently present with early, intermediate or late signs of encephalitis, and typically prove to have JE. Such trials might have significant limitations, but as current immunization campaigns begin to reduce the incidence of JE in India and other areas of Asia (www.path.org/je), it may become increasingly difficult to find suitable subjects for inclusion in carefully designed clinical trials. Testing in animal models will therefore become even more important as a means of establishing whether or not an antivirals is effective against JE, either to prevent the onset of symptoms or after the onset of encephalitis.

15. What is the future for JE antivirals?

Current efforts to control JE in India and other parts of Asia are reducing the problem of this disease, but the geographic range of the virus is still expanding. Thus, the burden of neurological sequelae remains heavy, justifying increased efforts to identify and develop antivirals that can prevent or mitigate CNS injury. Despite progress in developing antivirals against JE and other flaviviruses in the laboratory, for the immediate future there is little likelihood that safe, effective and affordable drugs will become available for use in poor countries. Looking further ahead, however, broad-spectrum antiviral agents that target both JE and a disease of interest to drug companies, such as HCV, and that could be produced cheaply by ensuring a larger demand, may prove to be the best hope. Failing this, antivirals are likely to be used only on a relatively small scale, as prophylaxis for persons visiting endemic areas and to treat severely ill patients.

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