Neurol J Southeast Asia 2003; 8 : 57 – 63

Enterovirus 71 in the Asia-Pacific region: An emerging cause of acute neurological disease in young children

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Abstract

Since its discovery in 1969, enterovirus 71 (EV71) has become recognised as a frequent cause of epidemics of hand-foot-and-mouth disease (HFMD) associated with severe neurological complications in a small proportion of cases. There has been a large increase in EV71 epidemic activity throughout the Asia-Pacific region since 1997. Recent HFMD epidemics have been associated with a severe form of brainstem encephalitis associated with pulmonary oedema and high case-fatality rates. The emergence of EV71 activity in the region has been associated with the circulation of several distinct genetic lineages that appear to be undergoing rapid evolutionary change. This review will present a synopsis of recent research into the epidemiology and evolution of EV71 in the Asia-Pacific region, the neurological diseases attributable to EV71 infection, the prospects for control of EV71 infections through public health interventions and the development of new antiviral agents and vaccines.

INTRODUCTION

Enterovirus 71 (EV71) belongs to the human enterovirus A species of the *Enterovirus* genus within the family *Picornaviridae*.[1] Virions consist of a non-enveloped capsid surrounding a core of single stranded, positive-polarity RNA approximately 7.5 kb in size. Since the initial description of EV71 in 1974[2], outbreaks of infection with this virus have occurred periodically throughout the world.[3-10] EV71 infection manifests most frequently as the childhood exanthem known as hand-foot-and-mouth disease (HFMD) and is considered to be clinically indistinguishable from HFMD caused by Coxsackievirus A16 (CA16). Molecular studies of the evolution of human enteroviruses have shown that EV71 and CA16 share a close genetic relationship, and, together with CA7 and CA14, form a distinct genetic sub-group within the human Enterovirus A species.[1] Despite the close genetic relationship between EV71 and CA16, EV71 has a propensity to cause neurological disease during acute infection[3,4], a feature not observed in CA16 infections. Children under five years of age are particularly susceptible to the most severe forms of EV71-associated neurological disease, including aseptic meningitis, brainstem and/or cerebellar encephalitis, and acute flaccid paralysis (AFP). The neurological complications of EV71 infection may occasionally cause permanent paralysis or death. Several large epidemics of severe EV71 infection in young children, including numerous cases of fatal brainstem encephalitis, have recently been reported from South East Asia[11-13], raising concern that both the prevalence and virulence of EV71 may be increasing. In this review, a synopsis of recent research into the epidemiology and evolution of EV71 in the Asia-Pacific region, the neurological diseases attributable to EV71 infection and the prospects for control of EV71 infections through public health interventions and the development of new antiviral agents and vaccines will be presented.

MOLECULAR EPIDEMIOLOGY OF ENTEROVIRUS 71

Our current understanding of the molecular epidemiology and evolution of EV71 has been defined by the publication of a seminal study by Brown *et al.*[14] This study compared the complete capsid protein (VP1) gene sequences of 113 EV71 isolates from around the world and demonstrated the development of three independent genetic lineages of EV71 (A, B, C) over a thirty-year period. Genogroup A includes a single virus, the prototype strain BrCr.[2,15] All other EV71 strains examined in the study belonged to either genogroup B or C, both of which were divided into two sub-lineages B1/B2 and C1/C2, respectively.

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Numerous reports on the molecular epidemiology of recent EV71 strains from the Asia-Pacific region have been published.[16-23] However, the use of different genome regions for analysis (5´ untranslated region, VP1 gene, VP4 gene) and the limited number of virus isolates examined in many of these studies prevented the development of a unified model of the molecular epidemiology of EV71 in the Asia-Pacific region.

Two recent studies have unified molecular epidemiological data from the Asia-Pacific region and provided a clear model of EV71 evolution.[24,25] This was achieved by analysing genetic data from large numbers of EV71 strains across wide geographic and temporal boundaries and by cross-referencing data derived from both VP1- and VP4-based genetic analyses. EV71 appears to have undergone rapid evolutionary change during circulation within the Asia-Pacific region in the past seven years. There appear to be two independent regions of EV71 circulation and evolutionary development: viruses belonging to genogroup B predominate in southeast Asia and viruses belonging to genogroup C predominate in northern Asia.[22-26] Three previously undescribed lineages of genogroup B (B3, B4, B5) have emerged in southeast Asia during in the past seven years – B3 in Malaysia, Singapore and Australia between 1997 and 1999, B4 in Malaysia, Singapore and Australia between 2000 and 2002 and B5 in Sarawak during 2003. Viruses belonging to genogroup C2, closely related to strains described by Brown *et al* [14], circulated widely in Japan and Taiwan between 1998 and 2000. Furthermore, two previously undescribed genetic lineages, C3 and C4, have been identified in northern Asia. Genogroup C3 was first identified in Korea during a HFMD epidemic in 2000[25] but appears to have circulated in mainland China as early as 1997.[26] Viruses belonging to genogroup C4 appear to have circulated in mainland China in 1998 and again in 2000.[26]

Despite the regular emergence of new genetic lineages of genogroups B and C, molecular genetic studies have not revealed specific neurovirulent genotypes associated with the severe and fatal cases of EV71 infection. Viruses belonging at least four different lineages (B3, B4, B5, C2) have been isolated from fatal cases throughout the region.

ENTEROVIRUS 71 AND NEUROLOGICAL DISEASE

Since it was first identified, EV71 has been recognised as highly neurotropic and associated with a diverse range of neurological diseases, such as aseptic meningitis, brainstem and/or cerebellar encephalitis, AFP and several post-infectious neurological syndromes.[27] The link between EV71 infection and AFP has been established by numerous studies over many years.[3,5,6,28-32] However, only a small number of studies have provided rigorous radiological[3,33,34] or histopathological[30,35] evidence for the induction of paralysis through infection and destruction of anterior horn motor neurons of the spinal cord - a process identical to that of poliovirus.[36] While it is clear that the pathogenesis of AFP in the Bulgarian EV71 epidemic[30,35] and in some cases of paralysis in Taiwan[37] was very similar to that of poliomyelitis, it is possible that EV71 may induce AFP by several mechanisms in addition to virus-mediated destruction of anterior horn motor neurons, and this is reflected in the more varied clinical presentation of EV71-associated neurological disease than that seen in poliovirus infections.

The most severe neurological manifestation of EV71 infection is brainstem encephalitis.[13,32,33,35,38-40] MR imaging and post-mortem studies indicated that the medulla oblongata, reticular formation, pons and midbrain structures were most frequently involved.[13,32,35,38] Children with this infection usually present with myoclonus, tremor, ataxia, nystagmus and cranial nerve palsies.[32,33,38] During the 1998 Taiwanese epidemic, brainstem encephalitis was classified into three grades (I, II, III) based on clinical criteria.[38] Grade I (mild) brainstem encephalitis was characterised by generalised myoclonus and ataxia, was associated with 100% recovery and only 5% of children developed permanent neurological sequelae. Grade II disease was associated with cranial nerve palsies in addition to myoclonus and ataxia; 100% of children with grade II disease recovered and 20% developed permanent neurological sequelae. Grade III disease was associated with a rapid onset of cardiopulmonary failure ("neurogenic pulmonary oedema"); approximately 80% of children with grade III disease died and all surviving children developed significant neurological sequelae.

Prior to the large epidemics in the Asia-Pacific region, only one case of EV71-associated brainstem encephalitis presenting with neurogenic pulmonary oedema had been described.[41] This case occurred in a 3-year-old girl from Connecticut, USA in 1995 and was fatal; EV71 was isolated from the CSF and spinal cord at post-mortem.[41] In retrospect, it is possible that the rapidly fatal cases

identified in the Bulgarian epidemic[30,31,35] may have been due to a similar syndrome.

The most striking clinical characteristic of type III brainstem encephalitis is its rapid progression and high mortality.[38,42-44] Typically, children develop tachycardia, tachypnoea and cyanosis between 2-5 days after the onset of fever, HFMD or herpangina.[13,45] The mortality from this condition is approximately 80% [33,38,43], with most children dying within 12-18 hours of the onset of illness.[42-44] Despite the high mortality associated with this condition, it has been suggested that mortality can be prevented through the provision of specialised paediatric intensive care support, although all survivors appear to be left with significant neurological sequelae.[46]

Several post-mortem studies of EV71-associated neurogenic pulmonary oedema have been published.[13,33,39,44,47] In each case, disease appears confined to the brainstem, with histological evidence of acute inflammatory encephalitis, isolation of EV71 or identification of EV71 antigen within neurons.[13,32,33,44,48] These studies support the hypothesis that the pulmonary oedema is of neurogenic origin and is secondary to autonomic dysfunction resulting from infection of specific regulatory structures within the brainstem. These findings are supported by neuroradiological evidence of brainstem pathology in many cases of neurogenic pulmonary oedema.[33,38] Post-mortem studies also consistently showed that the brainstem lesions included neutrophil and mononuclear cell inflammatory infiltrates.[13,33,38,44,47,49] These findings are supported by experimental infection of *Cynomolgus* monkeys with EV71, in which the development of paralytic disease was associated with inflammatory infiltrates in the spinal cord and medulla oblongata.[50-52] Thus, although EV71 is known to be cytopathic in cell culture[53], it is possible that inflammation contributes to the pathogenesis of encephalitis and neurogenic pulmonary oedema *in vivo*. Several clinical studies in support of this hypothesis have been published recently. A Taiwanese group showed that children with severe forms of EV71 encephalitis were significantly more likely to possess a specific cytotoxic T lymphocyte antigen haplotype (CTLA-4) than children who developed mild EV71 infections.[54] The authors suggested that children whose T cells expressed the CTLA-4 antigen developed cell-mediated immune responses to EV71 infection that predisposed them to the development of severe disease.[54] Other clinical studies have demonstrated a correlation between levels of certain inflammatory cytokines in serum and cerebrospinal fluid and the severity of EV71-associated encephalitis.[55-57]

PROSPECTS FOR CONTROL OF ENTEROVIRUS 71 INFECTIONS

There is currently no effective antiviral treatment for severe EV71 infections and a vaccine is not yet available. Thus, the only means to prevent EV71 infection is through avoidance of contact between infected and susceptible individuals. Realistically, this can only be achieved through infection control actions of limited efficacy, such as hand-washing and reducing contact between infected and susceptible people during epidemics. Indeed, if these actions are to have any effect, it is imperative that adequate surveillance of EV71 activity be maintained in the community to provide early warning of impending epidemics. Surveillance activities include clinical surveillance for HFMD and laboratory surveillance to identify EV71 and other neurotropic enteroviruses. In response to the increased prevalence of EV71 in the Asia-Pacific region, several countries have implemented clinical and laboratory surveillance to monitor EV71 activity.[25,58,59] In some instances, it has been claimed that these programs have provided information that, when acted upon by public health authorities, resulted in early control of EV71 epidemics and reduced the total number of cases of neurological disease.[58,59]

Although a number of promising antiviral agents with activity against enteroviruses are currently being developed, none are close to release, and their effect on EV71 replication is unknown. The "WIN" group of compounds is the most promising of these agents, several of which have undergone clinical trial.[60] Their antiviral activity is thought to be mediated by stabilisation of the viral capsid and the prevention of virus uncoating after receptor binding. The WIN compound pleconaril[61] has been found to provide significant therapeutic benefit in aseptic meningitis due to many enterovirus serotypes and to ameliorate coryza due to rhinovirus infection.[60,62,63] Pleconaril is currently undergoing clinical trial in the USA. Unfortunately, pleconaril has been found to have limited activity against EV71 at concentrations tested *in vitro* (D.C. Pevear, personal communication) and anecdotal information on its use in EV71-associated brainstem encephalitis has indicated poor responses to therapy.

As indicated previously, there is some clinical evidence that inflammation may contribute to the pathogenesis of EV71 encephalomyelitis[55-57,64], suggesting that anti-inflammatory agents or intravenous immunoglobulin (IVIG) may be of use in the management of this disease. However, no reports providing a clear demonstration of the efficacy of IVIG, steroid or non-steroid anti-inflammatory agents in EV71 neurological disease have been published to date. Furthermore, although IVIG was used extensively in the management of neurological disease cases during the Taiwanese[65] and Western Australian[32] epidemics, it was not associated with objective evidence of improvement in clinical outcome.

The success of both the formalin-inactivated and live attenuated vaccines in controlling epidemic poliomyelitis and in the eradication of poliovirus highlights the potential for control of EV71 epidemics by mass vaccination. A formalin-inactivated EV71 vaccine was developed in response to the Bulgarian epidemic in 1975[30,35] but was not used to control the epidemic and has not been used since. Furthermore, no data on the efficacy of the Bulgarian vaccine is available. A research group from Taiwan has recently reported on the development of two candidate EV71 vaccines: (1) a formalin-inactivated whole virus vaccine, and, (2) a subunit VP1 vaccine.[66] Both vaccine constructs were shown to be immunogenic in mice.[66]

A major barrier to the development of an EV71 vaccine is the lack of a suitable animal model for the rigorous testing of vaccine immunogenicity and efficacy. Laboratory mice are only susceptible to EV71 infection in the first four days of life and become completely resistant by six days of age.[67] However, a recent study has demonstrated the ability of EV71 to infect newborn mice by the oral route, resulting in the development of both encephalitis and a characteristic skin rash.[68] This study indicates that the newborn mouse model may be of considerable use for studying the molecular genetics of EV71 virulence. Furthermore, as noted above, the newborn mouse model has been used successfully in the initial screening of the immunogenicity of candidate vaccines.[66] Although *Cynomolgus* monkeys are susceptible to infection with EV71 and develop encephalomyelitis and poliomyelitis-like paralysis after subcutaneous[50] or intraspinal[51,52] inoculation, the high cost of purchase and maintenance of these animals presents a major barrier to their use in large-scale pathogenesis and vaccine efficacy studies.

CONCLUSION

There has been a significant increase in EV71 epidemic activity in the Asia-Pacific region during the past seven years. In addition, a new clinical manifestation of EV71 infection, a rapidly fatal syndrome of neurogenic pulmonary oedema associated with brainstem encephalitis, has been identified. Molecular genetic studies of EV71 isolates indicate that seven distinct viral genotypes circulated in Sarawak, peninsular Malaysia, Singapore, Vietnam, Korea, Japan, Taiwan, China and Australia between 1997 and the present. Unfortunately, these studies have not provided conclusive evidence for an association between particular viral genotypes and the development of brainstem encephalitis and/or neurogenic pulmonary oedema.

At present, the reasons for the emergence of EV71 as a cause of large epidemics of acute neurological disease in young children in the Asia-Pacific region remain elusive. In many ways the emergence of EV71 as a cause of large-scale epidemics of encephalomyelitis and other acute neurological diseases is reminiscent of the emergence of epidemic poliomyelitis in Europe and North America during the late nineteenth century and it has been suggested that EV71 may become the major infectious cause of acute neurological disease in the world following the eradication of poliovirus.[10,69,70] Thus, it is imperative that the medical and scientific communities prepare for such an eventuality in order to avoid the large-scale loss of life and human potential that resulted from the poliomyelitis epidemics of the twentieth century and that has already occurred as a result of the EV71 epidemics in our region. Exciting developments in the techniques of molecular biology over the past twenty-five years have provided us with powerful tools to combat epidemics of EV71. It behoves us to use these tools to prevent EV71 infection through the development of regional surveillance to predict impending epidemics and to develop vaccines to protect our children from the devastating neurological consequences of this disease.

ACKNOWLEDGEMENTS

Our studies of EV71 have been supported by grants from the Princess Margaret Hospital for Children Research Fund, the Royal College of Pathologists of Australasia, the National Health and Medical Research Council of Australia and the Wellcome Trust.

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