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Review

Neurological approaches for investigating West Nile virus disease and its treatment in rodents

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ABSTRACT

West Nile virus (WNV) has had a major public health impact since its emergence in the Western Hemisphere; in 2012, nearly 3000 cases of WN neuroinvasive disease were identified in the United States. The underlying mechanisms of WN neurologic disease can only be studied to a limited extent in patients, but can be investigated in much greater detail in animal models. In this paper, we describe how we and others have employed a variety of electrophysiological and neurological techniques to study experimental WNV infections in hamsters and mice. The methods have included electrophysiological motor unit number estimation; optogenetic photoactivation of the spinal cord and electromyography; plethysmography; measurement of heart rate variability as an indication of autonomic nervous system dysfunction; and an assessment of spatial memory loss using the Morris water maze. These techniques provide a more refined assessment of disease manifestations in rodents than traditional measurements of weight loss and mortality, and should make it possible to identify targets for therapeutic intervention and to directly assess the effects of novel treatments.

Contents

1. Introduction

Some signs and symptoms in human subjects that may be tentatively associated with neurological involvement or that are clearly associated with West Nile neurological disease (WNND) can also be observed in mice or hamsters (Table 1), the two rodent species suitable for WNV investigations. These rodent models have been valuable for understanding the mechanisms of neurological signs and symptoms in human subjects and how they might be managed or treated.

Most human WNV cases are subclinical, or develop a short-term febrile illness, which is referred to as WN fever (Bode et al., 2006; Hayes et al., 2005; Sejvar, 2007). Fever is often recognized to occur during viremia, but fever is also associated with generalized inflammation of the meninges. Interestingly, WNV-infected hamsters monitored continuously with radiotelemetry do not have a fever during the viremic phase, but can have a temperature spike at days 5–6 when viral induced meningitis is observed (Siddharthan et al., 2009; Wang et al., 2013a) (Table 1). These data suggest that WN fever in some cases might reflect neurological involvement, and not just the viremic phase. Having an animal model

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Table 1
Comparison of West Nile virus human neurological signs and symptoms[a] to those seen in mice and hamsters[b] (Adapted from (Morrey et al., 2004b)).

Human subject signs and symptoms	Mouse signs[b]	Hamster signs[b]	References
Fever (influenza-like illness, biphasic, chill)	ND	Fever with meningitis	Bode et al. (2006), Petersen and Marfin (2002), Wang et al. (2013a), Weiss et al. (2001)
Meningitis, encephalitis	Meningoencephalitis	Meningoencephalitis	Ben-Nathan et al. (1996), Camenga et al. (1974), Hunsperger and Roehrig (2006), Omalu et al. (2003), Sampson et al. (2000), Sejvar et al. (2003a), Steele et al. (2000), Weiss et al. (2001)
Headache	ND	ND	Asnis et al. (2000), Carson et al. (2006), Mazurek et al. (2005), Sejvar et al. (2003a)
Neck stiffness (nuchal rigidity)	ND	ND	Chapa et al. (2003)
Poliomyelitis, paralysis, paresis	Poliomyelitis	Poliomyelitis, paralysis, paresis	Doron et al. (2003), Fratkin et al. (2004), Hunsperger and Roehrig (2006), Jeha et al. (2003), Omalu et al. (2003), Sejvar et al. (2005, 2003a,b), Xiao et al. (2001)
Tremors, Parkinsonism	Tremors – rare	Tremors in extremities	(Carson et al., 2006; Morrey et al., 2004b; Sejvar et al., 2003a; Wang et al., 2013b)
Memory impairment	ND	Spatial memory deficit	Carson et al. (2006), Cook et al. (2010), Gottfried et al. (2005), Jeha et al. (2003), Lindsey et al. (2010), Mazurek et al. (2005), Smeraski et al. (2011)
Conjunctivitis, chorioretinitis	ND, no ocular exudate	ND, ocular exudate	Asnis et al. (2000), Khairallah et al. (2004)
Respiratory distress	ND	Lethal respiratory distress	Agamanolis et al. (2003), Betensley et al. (2004), Morrey et al. (2012), Saad et al. (2005), Wang et al. (2013b)
Death (<0.1%)	Death (80–100%)	Death (60–90%)	Lindsey et al. (2010), Morrey et al. (2004b), Xiao et al. (2001), Morrey et al. (2012), Oliphant et al. (2005)
Autonomic dysfunction	Not apparent	Non-lethal autonomic dysfunction	Fratkin et al. (2004), Leis and Stokic (2005), Wang et al. (2011, 2013a)
Gastrointestinal distress	ND	Duodenal EMG suppression	Mazurek et al. (2005), Wang et al. (2011), Weiss et al. (2001)
Cardiac arrhythmia			Bode et al. (2006), Wang et al. (2011)
Bladder dysfunction, urinary retention	ND	ND	Saad et al. (2005), Shpall et al. (2003), Wang et al. (2011)
Proprioception	ND	ND	Moon et al. (2005)
Confusion (altered mental status)	ND	ND	Jeha et al. (2003), Mazurek et al. (2005)
Coma	ND	ND	Sejvar et al. (2003a)
Backpain, myalagia, arthralgia	ND	ND	Jeha et al. (2003), Lindsey et al. (2010), Mazurek et al. (2005)

ND-Not determined.
[a] Not all subjects show all symptoms or signs.
[b] Alignment with human symptoms were subjective and may not correlate exactly.

for WNV fever might provide an opportunity to investigate the cause of WNV-induced fever and the neurological implications in human subjects.

A small subset of WNV patients develops more serious neurologic deficits (Table 1). Patients can present with meningitis symptoms, which include neck stiffness and light sensitivity (Bouffard et al., 2004; Omalu et al., 2003; Sampson et al., 2000; Sejvar et al., 2003a; Steele et al., 2000; Weiss et al., 2001). Inflammation of the meninges can be observed in the rodent models (Ben-Nathan et al., 1995; Camenga et al., 1974; Hunsperger and Roehrig, 2006), which suggests that they also get disease signs of meningitis, but efforts to observe these signs have not been undertaken, except for perhaps the detection of fever associated with CNS infection as described above (Wang et al., 2013a).

Encephalitis as an infection of the brain is a more serious development of WNND (Table 1). WNV-infected neuronal cells have been observed postmortem in the brainstem, which contains many vital nerve connections for motor and sensory systems from the main part of the brain to the rest of the body, and in the cerebellum involved in motor control in addition some cognitive functions. In rodent models, tissues collected at the time of death do not typically contain abundant WNV-infected cells due to prior clearance by the immune system, so it is not possible to understand viral tropism and pathogenesis without sampling tissues throughout the course of disease development (Siddharthan et al., 2009; Tesh et al., 2005). Herein lies the value of rodent models in that they have been used in temporal studies to determine that the virus can infect many areas of the brain and spinal cord and subsequently affect neurological functions.

Some WNV patients complain of confusion or altered mental status (Carson et al., 2006) (Table 1). In a retrospective study with 54 persons about a year and a half after acute illness, the study cohorts scored below the 15 percentile on some cognitive tests as compared to normative controls. (Sejvar et al., 2008). Further human studies should be done to confirm these results, but rodent models could also help to identify neurological mechanisms of cognitive deficits. The greatest density of lesions in WNV-infected hamsters is observed in the area of the prefrontal cortex (PFC) (Siddharthan et al., 2009), which plays a critical role in cognition and executive functions in humans and rodents. Extensive studies in the rat model have revealed that sub-regions of the PFC control distinct components of cognitive executive function (Chudasama and Robbins, 2006; Dalley et al., 2004). Additional WNV-induced lesions are also observed in the limbic system particularly with the hippocampus (Hunsperger and Roehrig, 2006; Siddharthan et al., 2009) and thalamus (Ali et al., 2005; Davis et al., 2006). Lesions in these anatomical regions might affect cognitive function via disturbance of connections between the PFC and the limbic system. Behavioral assays in rodents coupled with virological and histological assays could elucidate the effect that WNV might have on cognitive and executive functions.

Some WNV patients describe symptoms that may reflect a loss of proprioception (Moon et al., 2005) (Table 1), which is a declining sense of the relative position of neighboring parts of the body. The