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Experimental Zika virus infection induces spinal cord injury and encephalitis in newborn Swiss mice

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ABSTRACT

A widespread epidemic of Zika virus (ZIKV) infection was reported in 2015 in South and Central America, with neurological symptons including meningoencephalitis and Guillain-Barré syndrome in adults, besides an apparent increased incidence of microcephaly in infants born to infected mothers. It is becoming a necessity to have a trustworthy animal model to better understand ZIKV infection. In this study we used newborn white Swiss mice as a model to investigate the ZIKV strain recently isolated in Brazil. ZIKV was inoculated via intracerebral and subcutaneous routes and analysed through gross histopathology and immunohistochemistry. Here we demonstrated first that the intracerebral group (ICG) displayed severe cerebral lesions, with neuronal death, presence of apoptotic bodies, white matter degeneration and neutrophil perivascular cuffing. In the subcutaneous group (SCG), we observed moderate cerebral lesions, morphologically similar to that found in ICG and additional myelopathy, with architectural loss, marked by neuronal death and apoptotic bodies. Interestingly, we found an intense astrogliosis in brain of both groups, with increased immunoexpression of GFAP (glial fibrillary acidic protein) and presence of hypertrophic astrocytes. The spinal cord of subcutaneous group (SCG) exhibited reduction of astrocytes, but those positive for GFAP were hypertrophic and presented prolonged cellular processes. Finally significant lesions in the central nervous system (CNS) were present in newborn mice inoculated by both routes, but SCG method led to an important neurological manifestations (including myelopathy), during a longer period of time and appears for us to be a better model for ZIKV infection.

1. Introduction

Zika virus (ZIKV) is an emerging arbovirus of the *Flaviviridae* family, genus Flavivirus, that was initially isolated, in 1947, from a Rhesus monkey in the Zika forest, in Uganda. Subsequent isolations of this virus were obtained from *Aedes africanus* mosquitoes, implicating these insects as vectors (Dick et al., 1952a). Serological evidence of infection by ZIKV in humans was first obtained in the 1950s, in Africa (Dick et al., 1952b). After the first human ZIKV infection in 1964 (Simpson, 1964), sporadic cases were reported in sub-Saharan Africa and Southeast Asia. In 2007, an epidemic of ZIKV infection was reported in Yap, Island of Micronesia (Lanciotti et al., 2008), and, subsequently, caused major epidemics in French Polynesia, New Caledonia, the Cook Islands, and Easter Island in 2013 and 2014 (Ioos et al., 2014).

In 2015, a dramatic increase in reports of ZIKV infection occurred in the Americas, especially in Brazil, and Zika epidemics has been declared a Public Health Emergence of International Concern in February 2016 (Chowell et al., 2016). Clinical manifestations of ZIKV infection are, usually: fever, headache, arthralgia, myalgia, and maculopapular rash, a group of inespecific symptoms that hampers differential diagnosis (Mlakar et al., 2016). Although the disease is self-limiting, some cases of neurologic manifestations, microcephaly and the Guillain–Barré Syndrome, were described in Brazil during the epidemics (European Centre for Disease Prevention and Control, 2015). Recently, some studies have reported detection of this virus in fetal and newborn brain tissue of

Abbreviations: ZIKV, Zika virus; GFAP, glial fibrillary acidic protein; CNS, central nervous system; PND, post-natal day; PBS, phosphate-buffered saline; ICG, intracerebral group; SCG, subcutaneous group; CG, control group.

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patients presenting microcephaly (Martines, 2016; Martines et al., 2016), and experimental studies have demonstrated neurological disease in both newborn and adult mice due to Zika infection (Cugola et al., 2016; Lazear et al., 2016). Established small animals models are essential for understanding the pathophysiology of human ZIKV infection. Swiss mice intracerebrally inoculated with the ZIKV strain isolated from the Zika Forest in Uganda revealed central nervous system (CNS) lesions, especially neuronal necrosis in hippocampus and astroglial hypertrophy (Bell et al., 1971). However, so far, few studies showed histologic lesions in mice from the new Brazilian strain of ZIKV (Cugola et al., 2016), especially with immunohistochemistry caracterization.

Astrocytes are cells from white and grey matter of CNS, with an important role in many neurological processes, including infectious diseases. Reactive astrogliosis is a defensive response to pathological agents and it may be highlighted by GFAP profile, evaluated by immunohistochemistry in paraffin embedded tissues (Verkhratsky et al., 2015). NeuN is a neuron restricted protein, negative in nonneural cells, as glia of all types, and it permits identification of neurons (Edgar and Rosenblum, 2008). The association of these markers allows the distinction of cells involved in inflammatory process and neuronal death.

In this report, we described clinical, necropsy and microscopy findings in intracerebral and subcutaneous newborn Swiss mice by a strain of ZIKV isolated from the recent outbreak in Brazil. Also, we aimed to characterize astrocyte activity and neurons distribution in both inoculated groups through GFAP and NeuN immunohistochemistry and to distinguish the lesions between animals inoculated by subcutaneous and intracerebral routes.

2. Materials and methods

2.1. Animal experiments

We used a 10% ZIKV suspension (strain SPH 2015), obtained from the 7th passage in mouse brain, to inoculate newborn, one day old mice. This strain was first isolated from a serum sample of a Brazilian patient with febrile exanthematic disease diagnosed with ZIKV infection by a specific real time RT-PCR, as described by Cunha et al. (2016).

Fourteen PND0 (post-natal day 0) male and female Swiss mice were selected from outbred colonies maintained within Adolfo Lutz Institute. All animal experiments were approved by the institutional Animal Care and Use Committee (application # 05-2016) and were performed following the guidelines of Conselho Nacional de Controle de Experimentação Animal (CONCEA). All work with infected mice and potentially infectious materials derived from them was conducted at biosafety level 2 at the Virology Center Laboratory, Adolfo Lutz Institute. Biological materials were inactivated before removal, according to standard operating procedures approved by the institutional biosafety committee.

Eight PND1 animals were inoculated intracerebrally, in the right parietal lobe, or subcutaneously, in lumbar region, using a 26 gauge needle, with 20 μl of viral suspension in phosphate-buffered saline (PBS) + 5% bovine albumin + streptomycin/penicillin, without previous anesthesia, by an experienced veterinarian. All animals were observed daily until development of symptoms and kept with the mothers during the experiment. Six control animals received an equal volume of the same diluent through the same routes. After appearance of severe clinical signs, including important lethargy and paralysis, all inoculated mice were euthanized by cervical dislocation under deep sedation with isofluorane.

2.2. Light microscopic examination

Samples of major tissues were collected at necropsy of animals, including brain, spinal cord, cervical lymph nodes, heart, lung, liver, kidney, gonads, spleen, esophagus, stomach, duodenum, jejunum, ileum, and cecum. Sections of each tissue were fixed in 10% neutral buffered formalin, for at least 24 h. Tissues were processed routinely and histologic section stained with hematoxylin and eosin. Slides were evaluated and described in a masked manner by two veterinary pathologists, and evaluated also at fluorescence microscopy in dark field to confirm presence of apoptotic bodies (Stinchcombe et al., 1995). Lesions were classified

Table 1
Main histopathological findings in brain and spinal cord from Swiss mice inoculated with ZIKV by intracerebral and subcutaneous routes, classified by intensity. Control group has been omitted from the table due to absence of histopathological lesions.

Brain						
Group	Animal	Perivascular cuffing	WM vacuolization	Congestion/ Haemorrhage	Gliosis	Neuronal death
Intracerebral	1722	+++	+++	+/–	+	++
	1723	+++	++	++/–	++	+++
	1724	+	+	+/–	+++	++
	1725	***	***	***	***	***
Subcutaneous	1726	++	++	–/+	+	+
	1727	+	++	–/+	+	+
	1728	+	+	–	++	+
	1729	++	++	–/++	++	+++
Spinal Cord						
Group	Animal	Perivascular cuffing	WM vacuolization	Haemorrhage	Gliosis	Neuronal death
Intracerebral	1722	–	+	–	–	–
	1723	–	–	–	–	–
	1724	–	–	–	–	–
	1725	–	+	+	+	+
Subcutaneous	1726	–	–	–	–	+
	1727	–	–	–	++	++
	1728	–	–	–	+	+
	1729	–	+	–	+	–

***Not represented.