Investigating the Infection and Persistence of Sindbis Virus in Host Neurons

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INTRODUCTION

Sindbis virus, an Alphavirus in the *Togaviridae* family, is an enveloped, single-stranded positive-sense RNA virus. Found mostly in parts of Africa, Australia, Egypt, Philippines, and Northern Europe – it is known to cause Ockelbo or Pogosta disease [1]. This disease is characterized by the sudden onset of fever, headache, and arthralgia; followed by arthritis, rash, fatigue, and muscle pain. The symptoms are gone within 14 days, though cases have shown joint pain to last from 12 months to 2 and a half years [4]. Common to several other viruses, Sindbis is transmitted from birds (its reservoir) to humans via an arthropod vector, the mosquito [5]. The transmission and symptoms of Sindbis virus are well documented. Once inside a human host, however, much less is known. When Sindbis enters the body, its target is the nervous system. The mechanism of not only neuroinvasion, but neurovirulence and persistence is unknown. Both the virus and the host play important roles in the progression of a neurological, viral infection [2,6]. The aim of this study is to investigate the infection and persistence of Sindbis virus in an environment that replicates the neurological system of a rodent host using iCHIP (in-vitro Chip-Based Human Investigational Platform).

The multi-electrode array (MEA) on the iCHIP is used to detect signaling between the seeded neurons and to map the effect of the virus on them [3]. After detection, samples are taken at intervals and tested to observe persistence of both live virus and viral RNA. We hypothesize that the samples will show evidence of viable virus for the first couple weeks of sampling via a TCID50 assay, but then observe drop in viral population while the levels of viral RNA remain constant.

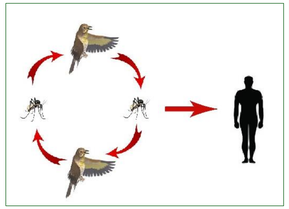


Fig. 1: An example model of the transmission of Sindbis virus between reservoir (birds), vector (mosquito), and incidental mammalian host (human).

STUDY OVERVIEW

- 16-electrode arrays were seeded with primary rat hippocampal cells and allowed to mature for 5 or 8 weeks (young and old neurons).
- Devices were infected with a strain of Sindbis (TEdsGFP) that allowed visualization of viral infection via florescent microscopy. **(Figure 2A & B)**
- 80 µL aliquots of media were removed at 2, 6, 13, and 23 days post-infection for the young neurons and 2, 8, 15, and 22 days post-infection for older neurons, then replaced with new media. 30 µL of each aliquot was used to isolate RNA via an Invitrogen™ TRIzol® extraction.
- Each RNA sample was converted to cDNA using the Invitrogen™ SuperScript® III First-Strand Synthesis System and used in PCR.
- Two PCRs were run using the cDNA product: The first was done using the Sigma-Aldrich© JumpStart™ REDTaq® ReadyMix™ and specific Sindbis virus primers, the PCR products produced were run on an Invitrogen™ E-Gel® to confirm the presence of viral RNA. **(Figure 4)**
- The second PCR utilized the Invitrogen™ Platinum™ Taq DNA Polymerase, paired with a Sindbis specific probe to indicate the viral titer in each sample. **(Figure 5)**
- A TCID50 assay was then performed on the positive samples by adding dilutions in triplicate to 96 well plates, pre-seeded with baby hamster kidney cells (BHKs).**(Figure 3A,B,C,&D)**
- The wells were examined over a period of 5 days to record any cytopathic effects (CPE) from the virus on the BHKs.
- The presence or absence of CPE was then used to determine when Sindbis was no longer viable and only viral RNA remained. **(Figure 6)**

Fig. 2A & B: Images of the iCHIP

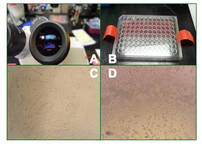
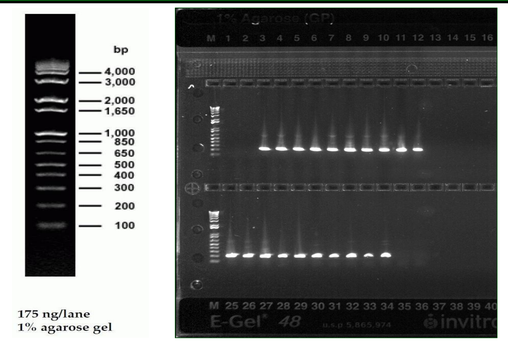


Fig. 3A,B,C,&D: A, A low powered light microscope was used to view and photograph the cells as the virus infected them. B, each viral sample was diluted and plated in triplicate on a 96 well plate. The 100, 1000, and 10000-fold dilutions were created for each sample. C, Image of un-infected, healthy BHK cells. D, Image of BHK cells showing CPE (Taken from Sample 572-5/20, 100-fold dilution).

RESULTS



175 ng/lane
1% agarose gel

Fig. 4: PCR products from the Sigma-Aldrich© JumpStart™ REDTaq® ReadyMix™ reaction run on an Invitrogen™ E-Gel®. The band at 220bp indicates the presence of Sindbis virus ' RNA. Lanes 1 and 2 show no band, indicating there is no viral RNA present in the sample. Lanes 35 and 36 were negative (cells only – no virus) and no template (cDNA) controls, respectively. Wells 1-12 and 25-36 were loaded with the samples shown by the chart in Fig. 5 (Below).

Well	Content	Sample	Biological Set Name	Cq
A01	Unkn	7-Jun	734	33.14
A02	Unkn	7-Jun	788	33.35
A03	Unkn	20-May	572	23.42
A04	Unkn	20-May	718	23.01
A05	Unkn	20-May	719	22.42
A06	Unkn	10-Jun	734	22.33
A07	Unkn	10-Jun	788	25.93
A08	Unkn	24-May	572	21.05
B01	Unkn	24-May	718	25.24
B02	Unkn	24-May	719	22.25
B03	Unkn	16-Jun	734	16.35
B04	Unkn	16-Jun	788	25.82
B05	Unkn	31-May	572	20.2
B06	Unkn	31-May	718	24.6
B07	Unkn	31-May	719	21.12
B08	Unkn	23-Jun	734	23.05
C01	Unkn	23-Jun	788	26
C02	Unkn	10-Jun	572	26.19
C03	Unkn	10-Jun	718	28.41
C04	Unkn	10-Jun	719	26.59
C05	Unkn	30-Jun	734	30.72
C06	Unkn	30-Jun	788	27.61
C07	Neg Ctrl	Blank		31.63
C08	NTC	No Template		30.45

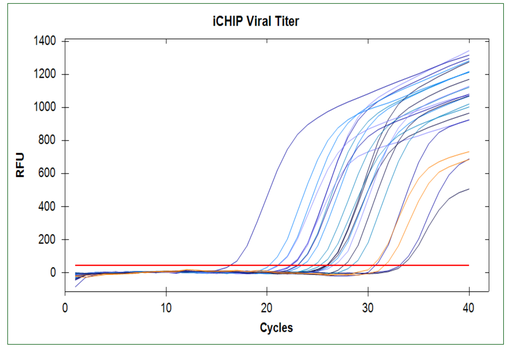


Fig. 5: Results of the RT-PCR using the Invitrogen™ Platinum™ Taq DNA Polymerase. The chart on the right shows each sample and its respective Ct value (based on the graph to the left). Values in orange are the negative and no template controls. The values in red indicate sample that had a higher Ct value than the controls (and were thus deemed negative for viral RNA). The one value in light green fell between the two controls. The TCID50 assay will be used to determine if that sample contains any viable virus.

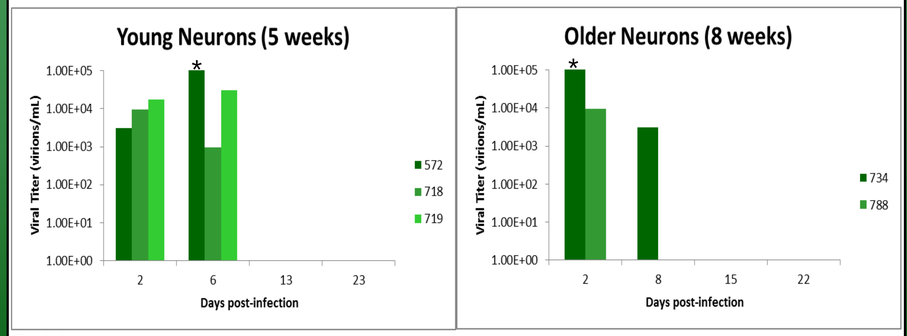


Fig. 6: Graphs showing the viral titer (virions per milliliter) for each sample device. No significant difference was seen between the infection rates of the young and older neurons. Columns marked with an asterisk (*) indicate samples that held a titer greater than 1x10^5.

CONCLUSION

Based on our findings, we were able to conclude that the amount of viable virus did decline as the infection continued, while the viral RNA levels persisted. This supports our hypothesis and other scientific findings that the Sindbis virus' RNA will persist after the infection has seemingly cleared [1]. Effects of remnant viral RNA has yet to be determined or linked to the long-term joint pain seen in patients after recovering from the infection. What is interesting to note is that while the infection of Sindbis (Fever, headache, muscle pain, rash, etc.) lasts for around 14 days, viable virus was only seen in our samples up to 8 days. More in-depth analyses of the RNA's impact on the body as well as the immune response to persisting RNA will need to be conducted to determine those effects. Our findings suggest that Sindbis' ability to persist in neurons, seemingly after resolution of the infection, indicate a level of immune-evasion and neurovirulence that we have yet to fully understand. Understanding the mechanisms of neuroinfections, like those of Sindbis, pose great benefit towards the protection and treatment of individuals susceptible to such diseases.

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