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Heparin prevents Zika virus induced-cytopathic effects in human neural progenitor cells

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ABSTRACT

The recent Zika virus (ZIKV) outbreak, which mainly affected Brazil and neighbouring states, demonstrated the paucity of information concerning the epidemiology of several flaviviruses, but also highlighted the lack of available agents with which to treat such emerging diseases. Here, we show that heparin, a widely used anticoagulant, while exerting a modest inhibitory effect on Zika Virus replication, fully prevents virus-induced cell death of human neural progenitor cells (NPCs).

1. Introduction

Zika virus (ZIKV) is a member of the *Flaviviridea* family comprising *ca.* 70 viruses sharing common structural features, and takes its name from the Zika Forest in Uganda where it was first isolated (Dick, 1953; Dick et al., 1952). It is the causative agent of an emerging infection arising from bites of the *Aedes aegypti* species for which, as yet, the full implications for human health remain unclear. Widespread infection and serious birth defects, including neurological diseases and microcephaly in Brazil have been reported (Rasmussen et al., 2016). In adults, ZIKV infection has been linked epidemiologically to increased prevalence of Guillain-Barré Syndrome (Cao-Lormeau et al., 2016; Parra et al., 2016). Thus, both *in utero* and adult, ZIKV-associated pathological conditions suggest that the virus could infect nerves (Bell et al., 1971) and neural cells (Garcez et al., 2016). Additional modes of transmission to mosquito bites have also emerged, including sexual transmission (D'Ortenzio et al., 2016; Foy et al., 2011; Musso et al., 2015) and blood transfusion (Motta et al., 2016). These could further expand the epidemic impact of ZIKV infection, increasing the need for an effective vaccine and antiviral agents with which to treat infected individuals.

Conventional antiviral drug-discovery pipelines involve complex, expensive, and time-consuming processes, militating against their rapid and widespread deployment, particularly in developing countries. One attractive alternative for combating emerging and rapidly spreading infectious diseases is drug repurposing (Cheng et al., 2016), the application of an existing licensed pharmaceutical agent for another medical need. This has already been proposed for the treatment of both hepatitis C (He et al., 2015) and Ebola virus (Sakurai et al., 2015) infections. Heparin, the widely used anticoagulant, has potentially attractive features including

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activity against attachment and entry of the enveloped viruses, human simplex (HSV) (Herold et al., 1996; Nahmias and Kibrick, 1964), human immunodeficiency (HIV) (Baba et al., 1988), SARS coronavirus (Vicenzi et al., 2004), and influenza (H5N1) (Skidmore et al., 2015). Here, we explore the effects of heparin on ZIKV infection in human neural progenitor cells (hNPCs) with particular regard to virus replication and induction of cytopathic effects (CPE) resulting in cell death.

2. Materials and methods

2.1. Cells

Human neural progenitor cells (hNPCs) were obtained by the reprogramming of human adult skin fibroblasts (Movement Disorders Bio-Bank, Carlo Besta Institute, Milan, Italy) and were reprogrammed (CytoTune-iPS 2.0 Sendai Kit, Life Technologies) to obtain human induced pluripotent stem cells (hiPSCs), then maintained in feeder-free conditions (mTeSR1 culture medium, Stem Cell Technologies), as reported (Orellana et al., 2016). For embryoid bodies (EBs), dissociated hiPSCs were seeded into low-adhesion plates in mTeSR1 medium supplemented with N2 (1:200), (ThermoFisher Scientific), human Noggin (0.5 μg/ml, R&D System), SB431542 (5 μM, Sigma), Y27632 (10 μM, Miltenyi Biotec) and penicillin-streptomycin (100 μg/ml, Sigma). For rosettes, EBs were plated after 10 days onto matrigel-coated plates (1:100, matrigel growth factor reduced, Corning) in DMEM/F12 (Sigma) with N2 (0.5%), non-essential amino acids (1%, ThermoFisher Scientific) and penicillin-streptomycin. After 10 days, cells were passaged with Accutase (Sigma) and seeded into matrigel coated-flasks in media containing DMEM/F12, N2 (0.5%), B27 (0.5%), (ThermoFisher Scientific), penicillin-streptomycin (1%) and basic fibroblast growth factor (FGF2) (20 ng/ml, ThermoFisher Scientific).

2.2. Viruses

The viruses employed were the historical ZIKV strain (MR766), (EVAg - European Virus Archive), or recent Brazilian strain (INMI-1; GenBank Accession # KU991811), isolated from an Italian citizen who had travelled in Brazil in January 2016. Both viral strains were

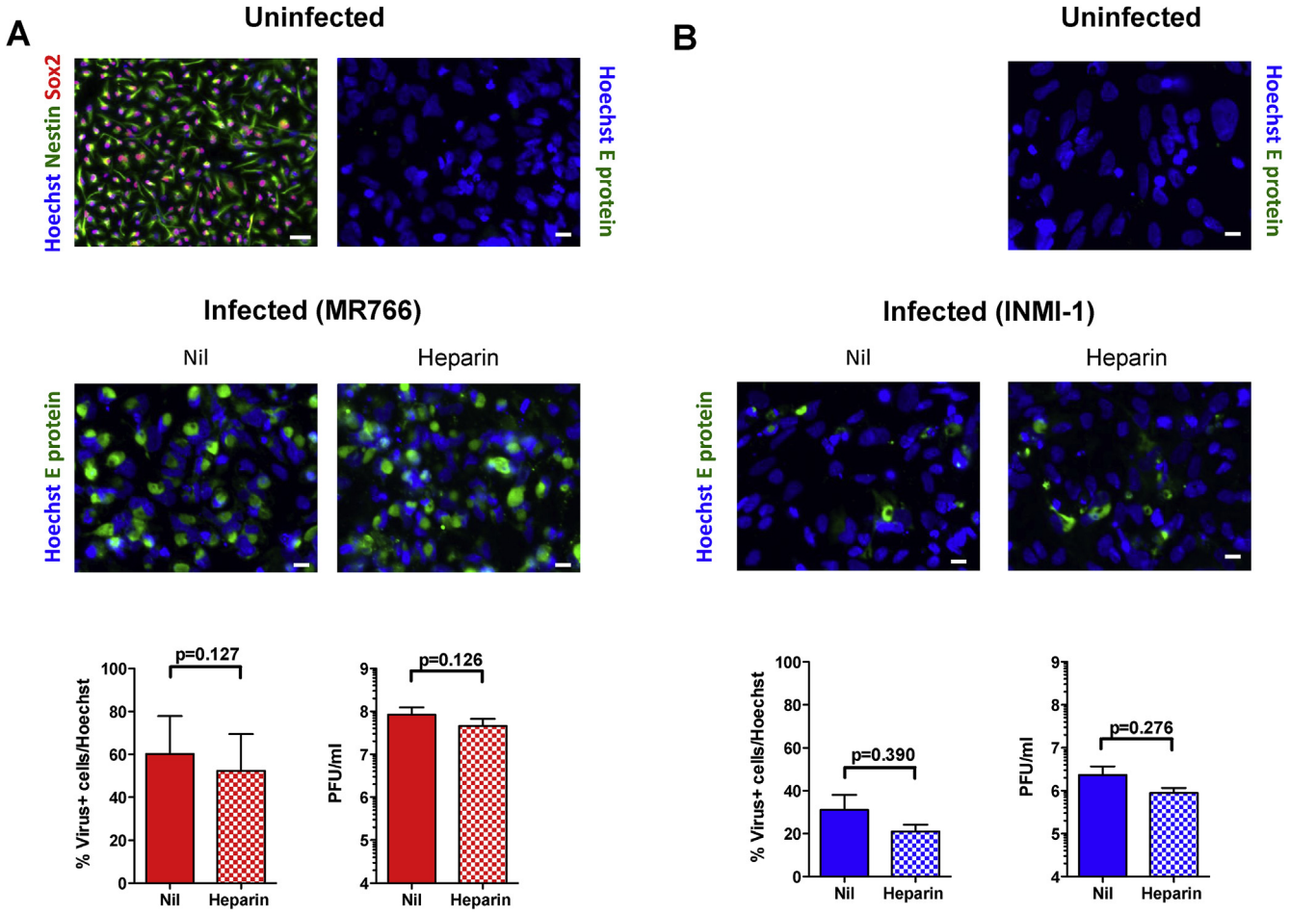

Fig. 1. Heparin does not inhibit ZIKV infection of hNPCs. **A.** Infection of hNPCs with the MR766 strain with (middle two panels) and without (upper two panels) heparin treatment (100 μg/ml). To determine that the cells were *bona fide* hNPCs, cells were stained with Sox2 (red), Nestin (green) and Hoechst (blue). Uninfected and infected cells were fixed after 3 days with paraformaldehyde solution and stained with a mAb specific for Flavivirus E protein (green). After PBS washes, cells were washed again, mounted and examined by microscopy. Quantification of infection efficiency and viral titers released into the culture supernatant are reported in the two lower panels (left and right, respectively). Scale bar of upper left panel: 20 μm, scale bar of remaining panels: 5 μm. Bar graphs indicate the mean ± SEM of 4 independent experiments. P values were calculated by Student's paired *t*-test. **B.** Infection of hNPCs by the INMI-1 strain, with (middle two panels) or without (upper panel) heparin treatment (100 μg/ml). Uninfected and infected cells were fixed after 7 days with paraformaldehyde solution and stained with a mAb specific for Flavivirus E protein (green). Quantification of infection efficiency and viral titers released into the culture supernatant are reported in the lower two panels (left and right, respectively). Scale bar: 5 μm. Bar graphs indicate the mean ± SEM of 2 fields including more than 1000 cells in 3 independent experiments. P values were calculated by Student's paired *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)