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Lack of efficacy of ivermectin for prevention of a lethal Zika virus infection in a murine system

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ABSTRACT

The antihelminthic drug ivermectin has been demonstrated to have antiviral activity against the Zika virus and other arboviruses in in vitro studies. The effectiveness of ivermectin for Zika virus infection, however, has never been studied in an animal model. In this study, ivermectin was found to be ineffective for prevention of a lethal infection with the Senegal strain of Zika virus in *Ifnar1* knockout mice. In view of several study limitations, evaluation of ivermectin's anti-Zika virus activity in other animal models and against other Zika virus strains would be desirable.

1. Introduction

A Zika virus pandemic has spread through Latin America and the Caribbean over the past few years (Morens and Fauci, 2017). Unlike other mosquito-borne viruses, Zika virus can be transmitted to the fetus in pregnant women and potentially result in serious birth defects, such as microcephaly. Zika virus can also be transmitted sexually and via laboratory accidents (Morens and Fauci, 2017). Zika virus may possibly be transmitted through blood transfusion and by organ transplantation (Morens and Fauci, 2017). No vaccine or drug treatment is available, although this is an area of intense investigation (Barrows et al., 2016; Xie et al., 2017).

Based on in vitro studies, a number of drugs have been identified that might have therapeutic value against the Zika virus (Barrows et al., 2016; Xie et al., 2017). One of these is ivermectin, a widely used drug to treat helminthic infections and scabies (Crump, 2017; Mastrangelo et al., 2012; Wagstaff et al., 2012). Although far from an ideal medication to treat Zika virus infections in humans since the drug does not cross the blood–brain barrier well (Crump, 2017), it is already undergoing a clinical trial in patients with another flavivirus infection, dengue (Croci et al., 2016). As no assessment of ivermectin's activity against Zika virus in vivo has been published (Abrams et al., 2017), we initiated a study of ivermectin in an animal model.

2. Methods

2.1. Reagents

Ivermectin (70288-86-7, Sigma) (molecular weight = 875.1 g/mol; molar concentration = 914.18 μmol/L) was dissolved in 100% dimethylsulfoxide (DMSO) at 800 μg/mL.

2.2. Cell culture

Vero (ATCC-CCL-81) cells were cultured in Dulbecco's modified eagle media with L-glutamine, 4.5 g/L glucose, and sodium pyruvate (MT10013CM, Fischer Scientific) at 37 °C in 5% CO_2. The culture medium was supplemented with 10% fetal bovine serum (ATCC-30-2020), 100 U/mL penicillin, and 100 μg/mL streptomycin.

2.3. Virus preparation

The Senegal strain of Zika virus was propagated in Vero cells. The culture medium contained secreted virions and was cleared to remove cellular debris by centrifugation at 1500 ×*g* at 4 °C for 20 min. Intracellular virions were also released by freezing and thawing, then similarly cleared of cellular debris, and combined with the secreted virions.

2.4. Animals

The animal protocol was approved by the Institutional Animal Care and Use Committee at New York Medical College, adhering to

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the National Institutes of Health recommendations for the care and use of laboratory animals. The *Ifnar1*$^{-/-}$ mouse line was obtained from Jackson Laboratories (B6.129S2-*Ifnar1*^{tm1Agt}/Mmjax). Five-week-old mice, each of which weighed approximately 15 g, were treated intraperitoneally with ivermectin 4 mg/kg (in ~70 μL of DMSO; 70 μL of 800 μg/mL of ivermectin corresponds to 4 mg/kg) or with an equal volume of DMSO alone 2 days preinfection with Zika virus, on the day of infection, and again 2 and 4 days after infection, totaling 4 treatments. Two days after receiving the initial dose of ivermectin or DMSO alone, and about 15 minutes after receiving the second dose of ivermectin or DMSO alone, the mice were injected with 1×10^3 plaque-forming units (PFU) of Zika virus into 1 foot pad. Infected mice were observed for morbidity and/or mortality until 10 days postinfection. A second control group consisted of 3 uninfected mice that received the same 4-dose treatment schedule of ivermectin and were evaluated over 20 days.

3. Results

Fifteen *Ifnar1* knockout mice were injected with 1×10^3 PFU of Zika virus into 1 foot pad. Eight mice were treated with 4 doses of ivermectin administered intraperitoneally, and 7 mice were treated with an equal volume of DMSO also given intraperitoneally. Treatment was initiated prior to inoculation of Zika virus to determine if ivermectin would prevent infection and to assess the antiviral activity of ivermectin before the Zika virus had crossed the blood brain barrier. All 15 mice appeared sick by day 3 postinfection, manifested by inactivity and shivering. Of the 7 control mice, 4 died on day 9 after infection and 3 on day 10. In comparison, 5 of the 8 ivermectin-treated mice died on day 8, and the other 3 died on day 10. In contrast, none of the 3 uninfected mice that were similarly treated with the 4-dose treatment schedule of ivermectin became ill, and none expired until they were euthanized on day 20 after receipt of the first dose of ivermectin.

4. Discussion

Ivermectin has been shown to inhibit Zika virus (Barrows et al., 2016) and many other arboviruses in vitro (Barrows et al., 2016; Mastrangelo et al., 2012; Tay et al., 2013; Varghese et al., 2016; Wagstaff et al., 2012). A potential viral target of ivermectin is inhibition of the flaviviral nonstructural (NS) protein 3 helicase enzyme (Mastrangelo et al., 2012). In addition, ivermectin has demonstrated antiviral activity against several RNA viruses by blocking the nuclear trafficking of viral proteins in the host cell through targeting the binding of viral NS5 with its nuclear transporter importin α/β (Tay et al., 2013; Wagstaff et al., 2012). However, despite the promising in vitro findings, ivermectin has never been tested against Zika virus in an animal model.

We investigated the efficacy of ivermectin in *Ifnar1* knockout mice, which are highly susceptible to Zika virus infections and have been previously used to study Zika virus pathogenesis (Lazear et al., 2016). In this study, the timing of administration of ivermectin was based on available but limited data that the serum half-life in rodents is approximately 24 h (Chiu and Lu, 1989; Conole et al., 2003). Therefore, administering the ivermectin at 48-h intervals is equivalent to redosing the medication after 2 half-lives. This dosing schedule is consistent with the timing of administration of other antimicrobials, such as vancomycin, in humans (Vancomycin [package insert], 2010).

Ivermectin failed to prevent infection. In addition, no difference in morbidity or mortality was observed between the control and ivermectin-treated infected mice, further documenting lack of efficacy in this animal model. The amount of ivermectin in milligrams per kilogram given per dosage in this study exceeded the United States Food and Drug Administration–approved dosage of ivermectin for humans, but higher doses have been studied clinically and were well tolerated (Guzzo et al., 2002). Furthermore, the mice did not die from drug toxicity. No overt sickness was noted for ivermectin-treated but uninfected mice through a 20-day period of observation.

One potential explanation for the lack of efficacy in this animal system is that the dosage of ivermectin administered may have been too low. In the in vitro study that found that ivermectin had activity against Zika virus, the drug level regarded as likely to be effective varied from 875 ng/mL to 8750 ng/mL depending on the cell line and the strain of Zika virus studied (Barrows et al., 2016). Although we did not measure blood levels of ivermectin in our study, a parenteral dose of 4 mg/kg should have resulted in a peak blood level of ivermectin that exceeded 875 ng/mL but would be less than 8750 ng/mL. We did attempt to investigate higher doses of ivermectin, first 8 mg/kg and then 6 mg/kg, in the *Ifnar1* knockout mice, but with the higher dosages, the treated uninfected mice rapidly became sick.

Other limitations of this study included use of only a single strain and only a single inoculum size of Zika virus, use of only a single animal model, use of a relatively small number of mice per group, failure to repeat the experiment to document reproducibility, failure to measure the viral load, failure to assess whether ivermectin caused immunosuppression of the mice, and failure to perform a pathological evaluation of the deceased mice to be certain they died because of Zika virus infection. Thus, additional studies on the efficacy of ivermectin in vivo may be warranted.

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Disclosures

Dr. Wormser reports receiving research grants from Immunetics, Inc.; Institute for Systems Biology; Rarecyte, Inc.; and Quidel Corporation. He owns equity in Abbott/AbbVie, has been an expert witness in malpractice cases involving Lyme disease, and is an unpaid board member of the American Lyme Disease Foundation. Other authors: none.

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