

Review Article

Indian J Med Res 138, November 2013, pp 762-765

Approaches to the treatment of disease induced by chikungunya virus

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Received October 15, 2013

Chikungunya virus, a re-emerging mosquito-borne alphavirus, causes fever, rash and persistent arthralgia/arthritis in humans. Severe outbreaks have occurred resulting in infections of millions of people in Southeast Asia and Africa. Currently there are no antiviral drugs or vaccines for prevention and treatment of chikungunya infections. Herein we report the current status of research on antiviral drugs and vaccines for chikungunya virus infections.

Key words Antivirals - arthralgia - chikungunya - CHIKV - vaccines

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne virus belonging to the Family, *Togaviridae*, and genus, *Alphavirus*. In this group of 29 viruses, six cause arthralgia and arthritis upon infection in humans. These six viruses are CHIKV, Ross River virus (RRV), Semliki Forest virus (SFV), o'nyong-nyong virus (ONNV), Sindbis virus (SINV) and Mayaro virus (MAYV). CHIKV was first isolated from a febrile patient in Tanzania in 1952¹. The name chikungunya means "that which bends up" and refers to the contorted posture adopted by infected patients. Symptomatic CHIKV infections are characterised by fever, rashes and arthralgia leading to arthritis. In the last 50 years there have been frequent outbreaks of CHIKV disease in Asia and Africa. In 2005-2006 an outbreak that occurred in La Réunion Island resulted in approximately 260,000 cases of CHIKV associated

fever with 237 deaths reported². Additionally, in 2006-2007 around 1.5 million cases of CHIKV disease were reported in India³. Through viral adaptation CHIKV has broadened its vector competence and therefore increased its potential to cause human disease. A single mutation, A226E in the envelope protein 1 (E1) of CHIKV, now allows *Aedes albopictus* as well as *Ae. aegypti* mosquitoes to transmit CHIKV⁴.

Currently there are no vaccines or antivirals for the prevention or treatment of CHIKV infections. Treatment is symptomatic with analgesics, antipyretics and non-steroidal anti-inflammatory agents. Passive immunization with human IgGs derived from CHIKV infected patients has been shown to be protective in mice against CHIKV challenge⁵. This approach may prove effective for the prevention and treatment of neonates who are at the risk of infection from viraemic mothers. However, this is not cost-effective and it is

critical to develop antiviral drugs and/or vaccines for the control of CHIKV infections.

Owing to the recent outbreaks of CHIKV in Asia, Africa and La Réunion island there has been a renewed interest in the development of antiviral drugs and vaccines to manage CHIKV infections. In this article, we report on the advancements made towards the development of anti-inflammatory agents, antivirals and vaccines for the efficient control of CHIKV infections.

Antiviral compounds

Chloroquine phosphate was reported to be effective in the treatment of chronic CHIKV arthritis⁶. However, in a clinical trial of chloroquine in CHIKV infected patients, no difference was observed between the placebo group and the treatment group⁷. Maheshwari *et al*⁸ have also reported that in a mouse model, chloroquine enhances alphavirus replication and aggravates disease pathogenesis. Ribavirin, an antiviral agent was used to treat CHIKV induced arthritis in a small cohort of patients and was found to be beneficial in resolving joint and soft tissue swelling⁹. Briolant *et al*¹⁰ have reported a synergistic effect of ribavirin and interferon- α in the inhibition of CHIKV and SFV replication in cell culture. Recently Rulli *et al*¹¹ have reported using bindarit, a small molecule anti-inflammatory drug, in a mouse model to effectively treat CHIKV induced arthritis. Bindarit has also been demonstrated to have significant effect in ameliorating RRV induced arthritis¹².

An additional 356 compounds (natural and clinically approved drugs) were initially screened for their antiviral effects using a CHIKV replicon cell line¹³. A recombinant CHIKV with *Rluc* (*Renilla* luciferase) fused with nonstructural protein 3 (CHIKV-*Rluc*) was constructed and used to validate the most active compounds from the initial screening studies. Out of 12 selected compounds, coumarin 30 was found to be the most potent in inhibiting the replication of CHIKV-*Rluc* virus (IC₅₀ value of 6.4 μ M). Arbidol, licensed for the treatment of influenza and other respiratory infections, was found to be effective in inhibiting CHIKV replication in Vero and MRC-5 cell lines (IC₅₀ <10 μ g/ml)¹⁴. Recently, screening of a highly purified natural compound library for inhibition of CHIKV replication identified 44 inhibitors¹⁵. Among the selected compounds, harringtonone, a cephalotoxine alkaloid, was found to be a potent inhibitor of CHIKV infection with an EC₅₀ value of 0.24 μ M. This drug was

found to inhibit the early stages of CHIKV replication. As this was also found to inhibit SINV replication suggesting the drug may be effective against other alphaviruses. In another study, an optimal homology model of CHIKV nonstructural protein 2(nsp2) protease was created using comparative modelling approach. Based on the active site of nsp2 model, a virtual screening was performed with commercially available compounds subsequently 26 compounds were evaluated for the anti-CHIKV activity in a cell culture assay¹⁶.

Vaccines

Several approaches have been undertaken to develop an effective vaccine against CHIKV infections. Levitt *et al*¹⁷ described the development of an attenuated strain of CHIKV (181/25) obtained by serial passage in MRC-5 cells. This vaccine candidate was tested in both mice and non-human primates and was found to protect the animals against challenge with parent virus. However, in Phase II trials the vaccine caused mild transient arthralgia in some of vaccinees¹⁸. Recent attempts to develop a CHIKV vaccine have used formalin inactivated vaccine¹⁹, virus like particles (VLPs)²⁰ and DNA vaccines^{21,22}. Immunization with VLPs and DNA vaccines resulted in immunogenicity and protected the mice and non-human primates against subsequent challenge with CHIKV.

Chimaeric viruses containing Venezeulean equine encephalitis virus (VEEV) or Eastern equine encephalitis virus (EEEV) or SINV non-structural proteins with CHIKV structural proteins elicited neutralizing antibodies and protected the mice against CHIKV challenge²³. However, these viruses still had the ability to infect mosquitoes and attenuation was dependent on an intact interferon response in mice. Another chimaeric vaccine using VEEV strain TC-83 and encephalomyocarditis virus (EMCV) IRES (internal ribosome entry site) in the subgenomic promoter was developed to reduce the transmission to mosquitoes. The EMCV IRES sequence cannot efficiently drive translation in arthropod cells. This vaccine was poorly immunogenic and lacked neutralizing antibody response²⁴. Recently Chattopadhyay *et al*²⁵ developed a chimaeric vaccine using a vesiculostomatitis virus (VSV) backbone and CHIKV structural proteins (VSV Δ G-CHIKV). This VSV Δ G-CHIKV chimaeric virus induced a good neutralizing antibody response and protected mice against CHIKV infection.

Plante *et al*²⁴ designed a vaccine by introducing the EMCV IRES sequence into the CHIKV subgenomic promoter. This reduced the replication of the vaccine strain in mosquitoes and was highly immunogenic in mice. This vaccine is now undergoing preclinical trials in non-human primates.

Short interfering RNA (siRNA) and short hairpin RNAs (shRNA)

siRNAs and shRNAs against envelope protein E1, nsP3, capsid and nsP1 proteins of the CHIKV have been used to inhibit the replication of CHIKV in mammalian cells with promising results^{26,27}. Furthermore, *in vivo* studies have shown that shRNA E1 offers a strong and sustained protection against CHIKV infection in suckling mice.

Other therapeutic options

Studies on the role of mannose binding lectin (MBL) in RRV-induced arthritis in mice²⁸ suggest that MBL may be a target for therapeutic intervention in the treatment of alphavirus induced arthritis/myositis. Inhibitors targeting the MBL pathway of complement may be useful in the treatment of alphavirus induced diseases. Additionally, a peptide corresponding to the autophagy inducing peptide, beclin, has been shown to be effective against CHIKV infections in cell culture and in mice²⁹.

A recent study on gene profiling has revealed a significant overlap of differentially expressed genes involved in the inflammatory processes of both CHIKV-induced arthritis and rheumatoid arthritis (RA). Thus, new drugs being developed for RA might also be beneficial for the treatment of alphavirus induced arthritis³⁰.

Conclusions

Chikungunya fever is a global health problem and several outbreaks have occurred in the last 50 years in Asia and Africa. Recent outbreaks in La Réunion and Italy have raised the awareness of the need to develop effective antiviral drugs or vaccines against CHIKV. Although many antiviral compounds have been shown to be effective in cell culture, very few compounds have been evaluated in animal models. Intensive studies are needed to evaluate the effect of appropriate compounds in animal models and humans.

Acknowledgment

This study was supported by a grant from the Australian National Health and Medical Research Council (NHMRC Project Grant 508600).

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