IJPSR (2020), Volume 11, Issue 12 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 December 2019; received in revised form, 07 April 2020; accepted, 10 April 2020; published 01 December 2020

RECENT ADVANCES TOWARDS THE DEVELOPMENT AND APPLICATIONS OF NOVEL THERAPEUTICS AGAINST CHIKUNGUNYA VIRUS INFECTIONS

S. Roy [*1], A. Bhattacharjee [2], L. Roy [3] and S. Das [4]

Department of Biotechnology [1], St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata - 700016, West Bengal, India.
Department of Biotechnology [2], National Institute of Technology Durgapur, Durgapur - 713209, West Bengal, India.
Department of Microbiology [3], Sarsuna College, 4/HB/A, Ho Chi Minh Sarani, Sarsuna Upanagari, Kolkata - 700061, West Bengal, India.
Department of Microbiology [4], Peerless Hospital & B.K Roy Research Centre, Kolkata - 700094, West Bengal, India.

Keywords:

Chikungunya infection, Novel chemical therapeutics, Immunotherapies, vaccines, Homoeopathic medicines

Correspondence to Author:
S. Roy

Assistant Professor,
Department of Biotechnology,
St. Xavier's College (Autonomous),
30, Mother Teresa Sarani, Kolkata - 700016, West Bengal, India.

E-mail:souvikroybiotech@sxccal.edu

ABSTRACT: Chikungunya infection, caused by the flavivirus Chikungunya, spreads from one person to another by two species of *Aedes* mosquitoes, *i.e.*, *Aedes albopictus* and *A. aegypti*. There is a rapid global emergence and resurgence of Chikungunya infections in India and the Indian Ocean Islands, making it a public health emergency around the globe. There are numerous strategies being adopted by this arbovirus to evade host immune defences, and as such specific preventive and therapeutic interventions to this fatal and incapacitating disease are still unavailable at large. Hence, under this grave global epidemiological scenario, the development of novel therapeutics against Chikungunya infections in humans is an absolute mandate now. Fortunately, in recent years, experimentations toward new approaches for the prevention and treatment of Chikungunya infections are already underway, with significant success being slowly achieved at the preliminary level of laboratory-based pilot-studies. In this article, we review some of these important advancements achieved in the development of novel chemical therapeutics, immunotherapies, vaccines, and complementary & alternative medicines against Chikungunya virus infections that have shown much promise and can hopefully open untraversed avenues before pharmacists and other health care professionals in the treatment of Chikungunya.

INTRODUCTION: Diseases caused by pathogenic viruses are not only fatal to humans but can be life-threatening under conditions of extreme immunosuppression, secondary pathogenic infections, improper or abruptly-truncated course of medication, poor sanitation, malnutrition, infancy and old age.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(12).6503-13

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(12).6503-13

Flaviviruses, including Yellow fever virus, Chikungunya virus, Dengue virus, Japanese Encephalitis virus, Tick-borne Encephalitis virus, and West Nile virus, are some of the globally important viral pathogens of humans.

Chikungunya is a severe febrile infection caused by the Chikungunya virus (CHIKV) which is an arthropod-borne virus (arbovirus) that circulates within a number of animals, including birds and rodents [1]. CHIKV is a member of the genus Alphavirus in the family Togaviridae that is transmitted to humans primarily through the bite of infected mosquitoes [2]. This arthritogenic virus usually spreads *via* two species of mosquitoes as

the vectors of the disease – *Aedes albopictus* and *A. aegypti*, and has created havoc in the tropical and subtropical regions of the world, particularly in Africa and Asia [3]. The main symptoms of Chikungunya are an abrupt onset of fever with severe back and small joint pain that manifests two to twelve days after the initial exposure to the virus, and debilitating arthritis, called Post-Chikungunya Chronic Arthritis (PCCA), which is associated with crippling pains that persist for weeks and even years [3]. Other symptoms include myocarditis, headache, pain in muscles, joint swelling, skin rashes and sleeping disturbances [3]. Severe CHIKV infections involving the central nervous system (CNS) have been recently reported from neonates as well as from adults [3]. Unfortunately, the pathophysiology of CHIKV infections and the immunopathologic basis for the severity of this disease are still obscure despite mammoth progress in medical science and research [3]. For individuals with a weakened immune system, the fever may even lead to death [3]. Diagnosis of chikungunya fever is usually done by either testing the patient's blood sample for the virus's RNA, or for the detection of antibodies to the virus [4].

Need for the Development of Novel Therapeutics against CHIKV Infections: Although many other alphaviruses have all been studied in detail, very less information is available regarding the life cycle, pathogenic behavior, and disease biology of CHIKV till date. Until recently, Chikungunya was a little known disease, the virus of which re-emerged in 2005–2006, leading to major epidemics on the Indian Ocean Islands and in South-East Asia, and subsequently spreading itself to temperate regions, thereby drawing much attention of the scientific community due to its explosive onset, extensive geographic distribution and a large number of cases of morbidity [5].

CHIKV has gradually gained great prominence as a global, re-emerging pathogen over the past two decades, progressing from sporadic, remote outbreaks to explosive epidemics worldwide, particularly in the African and Asian countries [5]. CHIKV is also believed to have acquired biologically significant mutations over years of its evolution, widening its geographic reach and preys [5]. Hence, over the past few years, there has been a multidisciplinary approach by scientists aimed at deciphering the clinical, physiopathological, immunological and virological features of CHIKV infections in humans [5].

According to the recent World Health Organization (WHO) reports, CHIKV infection is now a public health emergency in more than 100 countries, and 1.5 million people (over 38% of the world's population) across the globe are at high risks for contracting the disease for which registered antivirals are still unavailable [5]. As with most other mosquito-borne viral diseases, effective control of CHIKV infection depends on diminishing the mosquito populations and cutting off their every possible contact with man[5]. But with CHIKV, this approach has majorly failed in most of the locations targeted. Progressively increasing incidences of CHIKV infections globally, its co-infections with Zika/Dengue virus, and an overall failure in developing specific antiviral chemicals or effective vaccines against CHIKV have worsened the present situation manifold [5]. Also, a large number of people reside in high risk-prone zones for CHIKV without any proper access to vaccination, and unfortunately, the number of such individuals is continuously increasing in huge numbers [5]. The epidemiological situation is thus highly grave. Because of this fact, the CHIKV has adopted various strategies over the course of its evolution to overpower the host immune system.

An important one of these viral defense strategies is stopping the virus-infected human cells from sending out chemotactic signals to activate the adaptive branch of their immune system [6]. Consequently, CHIKV-mediated morbidity and mortality are on the cards now before us. This makes the search and development of novel therapeutics against CHIKV infections in humans an absolute need of the hour.

Diagnosis of CHIKV Infections Prior to Therapy: Diagnosis of CHIKV viral infection includes isolation and propagation of the virus in cell culture, followed by identification of the viral RNA by the conventional reverse transcriptase (RT)-PCR or modern real-time PCR, and detection of antibodies (IgM/IgG) to the virus by immunoassays and immunofluorescence (IFA) studies [7].

The CHIKV isolation is performed from serum samples collected from the patient within the first 7-8 days of illness[7]. Insect and mammalian cell lines like C636 and Vero respectively serve as important means for isolation and propagation of the virus in the laboratory [7]. The cultured virus can then be confirmed by RT-PCR or immuno-technique-based assays [7]. Traditional RT-PCR and recently-developed real-time PCR assays have been used for this purpose, targeting the structural (envelope) and the non-structural genes of CHIKV [7]. RT-PCR can be used for early identification of the CHIKV infection before the detection of the antibodies, *i.e.*, within the first 7–10 days of infection [7]. DNA sequencing, coupled with the RT-PCR technique, can be used to identify the corresponding genotype [7]. The modern real-time PCR technique is used to determine the viral load in the clinical samples as well as infected tissues from animals [7]. A real-time RT-PCR has a 10-fold higher sensitivity than the conventional RT-PCR in this regard [7]. The enzyme-linked immunosorbent assay (ELISA) for detection of anti-CHIKV IgM or IgG, and the indirect immunofluorescent assay (IFA) play a vital role in the detection of patient antibodies to the CHIKV [8]. Selected flavonoids like baicalein, fisetin, and quercetagetin displayed potent inhibition of CHIKV infection interfering binding to Vero cells [9]. Flavonoids also affect CHIKV RNA production and viral protein expression [9]. The sensitivity and specificity of serological assays for detection of CHIKV are however, poorly established, as false-positive results occasionally arise due to possible cross-reactivities with antibodies to Dengue or other arboviruses [10].

Taken into account there are limitations with the detection of the CHIKV infection by IgM-capture ELISA, recently a study has been conducted that tried to improve the ELISA by developing antigen for the assay using Eilat virus instead of the CHIKV[10]. The antigen that was developed in that study does not require biosafety containment facilities and can be used for ELISA detection without inactivation[10]. However, it requires proper testing and correct evaluation before its successful implementation on clinical samples [10].

Novel Strategies using Therapeutic Chemicals against CHIKV Infections: The absence of an effective and recommended antiviral therapy and a suitable vaccine makes dealing with chikungunya disease a great challenge before those involved in public health sectors. Although no scientifically-proven antiviral treatment against CHIKV infections is currently available for *in-vivo* applications, clinical trials by scientists all over the world have shown several medications to be effective against CHIKV at least *in-vitro* [11].

Recent breakthroughs in CHIKV-related research have made remarkable strides in the tough battle of the health community against this infectious disease. Recent reports are there of many promising chemicals against CHIKV, which need further clinical evaluation and increased availability before getting approved for therapeutic usage. Some of these chemicals with unique anti-CHIKV properties are discussed as follows:

1. 6-azauridine: 6-azauridine is a synthetic triazine analogue (N-glycosyl-1,2,4-triazine) of uridine. It has a significant role to play as an anti-metabolite, an anti-neoplastic agent, and a drug metabolite. It was found to inhibit *de novo* pyrimidine synthesis and DNA synthesis in the virus, converting intracellularly into mono-, di-, and triphosphate derivatives, which incorporate into the viral mRNA in order to inhibit protein synthesis [12]. This chemical inhibited CHIKV replication and was more effective against CHIKV as compared to the traditional antiviral ribavirin [12]. Antiviral activity of 6-azauridine was estimated by the reduction of the cytopathic effect (CPE) of each alphavirus on infected Vero cells and by a virus titer reduction [12]. It has been found that 6-azauridine, IFN-α2b, glycyrrhizin, and ribavirin cause a concentration-dependent reduction in the virus yield with CHIKV [12].

2. IFN-α2b and ribavirin in combination: Interferon-α2b is produced by host leukocytes and is a member of the Interferon (IFN) family. IFN-α is mainly involved in host innate immune response against a wide variety of viral infections. It contains four highly conserved cysteine residues, which form two disulfide bonds, one of which is necessary for its biological activity. IFN-α2 has three acid-stable forms (a, b, and c). Ribavirin, also known as tribavirin, is a synthetic guanosine nucleoside and a potent antiviral agent widely used

to treat Rous Sarcoma Virus (RSV) infection, Hepatitis C infection, and some viral hemorrhagic fevers. It interferes with the synthesis of viral mRNA so that subsequently, no proteins are synthesized from them. A combination of IFN-α2b and ribavirin has a sub-synergistic antiviral effect on CHIKV, and should be evaluated even more for its potency to treat CHIKV infections [12].

3. Lobaric Acid: CHIKV has a positive (+) strand RNA genome that requires a 5' cap to direct the translation of the viral polyprotein, and to prevent degradation of the viral RNA genome [13]. Formation of this 5' RNA cap is mediated by the CHIKV protein nsP1 [13]. Viruses with altered or reduced nsP1 activity are unable to effectively replicate in the host cell, suggesting that nsP1 can be hailed as an effective target for anti-CHIKV drugs [13]. Lobaric acid is a depsidone metabolite with antiviral and enzyme inhibitory activities that is derived from Stereocaulon lichen species. This naturally occurring carboxylic acid was found to inhibit CHIKV nsP1 GTP binding and guanylation, and attenuate viral growth *in-vitro* within both 24 h and 48 h post-infection periods in hamster BHK21 and human Huh 7 cell lines [13]. These observations however, necessitate further exploration of CHIKV nsP1 as a drug target by suitable clinical trials.

4. [1,2,3] Triazolo[4,5-d]pyrimidin- 7(6H)- ones: The compound [1,2,3]Triazolo[4,5-d]pyrimidin-7(6H)-ones are potent, selective inhibitors of CHIKV replication that has played a pivotal role in the inhibition of the *in-vitro* guanylyl-transfer activity of CHIKV nsP1, as determined by Western blot technique, using an anti-5' cap antibody [14]. This compound has shown antiviral activity against different CHIKV isolates in very low concentration range (at the level down to μM), the data validated from both virus yield reduction and virus-induced cell-killing inhibition assays [14].

5. Inhibitors of nsP2 protease: The CHIKV nsP2 protease is one of the most vital components of viral replication that plays an important role in the cleavage of the polyprotein precursors needed for the viral replication process to initiate [15]. Very recently, this protease is gaining importance as a potential drug target against CHIKV. Based on the recently determined crystal structure of the CHIKV nsP2 protease, multiple studies have been performed that have identified potential inhibitors of this alphavirus using structure-based approaches together with molecular docking, virtual screening, and molecular dynamics simulations [15]. The results of these experiments have provided sufficient information that would help in the development of inhibitors for CHIKV.

6. Suramin: Suramin contains six aromatic systems in the form of four benzene rings, sandwiched by a pair of naphthalene moieties, together with four amide functional groups (in addition to the urea) and six sulfonic acid groups. Suramin treatment reduces the viral burden in CHIKV-infected mice through alleviation of CHIKV-induced foot swelling [16]. Suramin treatment not only substantially decreased viral loads, but it also significantly ameliorated acute foot lesions in mice [16]. Additionally, Suramin treatment markedly restores cartilage integrity and reduces the number of IHC positive chondrocyte in mice infected with CHIKV strains 0810bTw and 0706aTw [16]. Suramin possibly interferes with (re)initiation of RNA synthesis, and also interferes with a post-attachment early step in infection, most possibly the entry of the virus into animal cells [16]. Experiments that were designed to test the antiviral effect of suramin-containing liposomes also yielded promising results [16].

7. Picolinic acid: It is known that protein-protein interactions (PPIs) of the transmembrane glycoprotein E2 with the hydrophobic pocket on the surface of capsid protein (CP) play a critical role in an alphavirus life cycle, including that of CHIKV [17]. The binding of picolinic acid (PCA), a derivative of pyridine with a carboxylic acid substituent at the 2-position, to the conserved hydrophobic pocket of CP of CHIKV was analyzed by molecular docking, surface plasmon resonance, isothermal titration calorimetry, and fluorescence spectroscopic studies, and it was found to inhibit CHIKV replication in infected Vero cells by reducing viral mRNA and viral load as assessed by qRT-PCR and plaque reduction assay respectively [17].

8. Arbidol: Arbidol (ARB/umifenovir) is a broad-spectrum antiviral agent approved in some countries for prophylaxis and treatment of influenza. This compound has shown prominent

activities against many RNA and DNA viruses, and the mode of action is based primarily on impairment of some critical steps in virus-host interactions. *In vitro* antiviral activity of arbidol has recently been reported against CHIKV [18]. This compound inhibited CHIKV propagated into immortalized Vero cells and primary human fibroblasts (MRC-5 lung cells) at very low concentrations [18]. Furthermore, characteristics of ARB-resistant mutants, including the mechanism of its resistance, are also being studied to understand the molecular basis of ARB activity [18].

9. Abamectin and Ivermectin: Abamectin and Ivermectin are fermentation products generated by a soil-dwelling actinomycete *Streptomyces avermitilis* that are anti-CHIKV in their mode of action. These inhibited CHIKV replication in a dose-dependent manner [19]. Abamectin and ivermectin reduced the synthesis of CHIKV genomic and antigenomic viral RNA, as well as downregulated viral protein expression much [19]. Time of addition experiments also suggested that they inhibit the replication phase of the viral infectious cycle [19].

10. Berberine: Berberine is a non-toxic, plant-derived, isoquinoline alkaloid that can control CHIKV replication in a dose-dependent manner and has enough potential to be a promising antiviral chemical [20]. Berberine was found to be highly effective in multiple cell types against a variety of CHIKV strains and also at a high multiplicity of infection (MOI), thus establishing the potential of berberine as an anti-CHIKV drug [20]. A human phosphokinase assay revealed that CHIKV infection specifically activated the major mitogen-activated protein kinase (MAPK) signaling pathways, extracellular signal-related kinase (ERK), p38, and c-Jun NH_2-terminal kinase (JNK), which are crucial to the generation of progeny virions [20]. Upon berberine treatment, this virus-induced MAPK activation was significantly reduced [20]. The *in-vivo* efficacy of berberine was also determined in a mouse model, and a significant reduction of CHIKV-induced inflammatory disease was reported [20].

11. Curcumin: Several compounds extracted from spices and herbs often show potential antiviral effects *in-vitro.* One of the most popular of these, curcumin, is a bright yellow chemical produced by turmeric (*Curcuma longa*) plants, a member of the ginger family, Zingiberaceae. Chemically, curcumin is a diarylheptanoid, belonging to the group of curcuminoids, which are natural phenols giving turmeric its bright yellow color. It is a tautomeric compound existing in keto form in water and enolic form in organic solvents. It is sold as a herbal supplement, cosmetics ingredient, food flavoring, and food coloring. This vital component of turmeric shows antiviral properties against several viruses, including Zika virus and CHIKV [21]. Both viruses responded to treatment with up to 5 μM curcumin, without impacting cellular viability much [21]. It was observed that direct treatment with curcumin reduced infectivity of virus in a dose- and time-dependent manner for these enveloped viruses [21]. Similarly, derivatives of curcumin also exhibited antiviral activity against such enveloped viruses [21]. Further experimentations revealed that curcumin interfered with the binding of the CHIKV viruses to cells in a dose-dependent manner, maintaining the integrity of the viral RNA [21].

12. Luteolin and Apigenin: Luteolin is a naturally-occurring flavonoid with significant anti-inflammatory, anti-oxidant, apoptosis-inducing and chemopreventive properties. It is an anti-neoplastic agent that induces autophagy in leukaemia cells, and also has a role as a metabolite. Upon administration, luteolin scavenges free radicals, protects cells from reactive oxygen species (ROS)-mediated damage, and induces direct cell cycle arrest and apoptosis in tumor cells. This inhibits tumor cell proliferation and suppresses metastasis. Apigenin, a conjugate acid of an apigenin-7-olate, on the other hand, is a trihydroxyflavone that is a flavone substituted by hydroxy groups at positions 4', 5, and 7. Luteolin and apigenin-rich ethanolic fractions from *Cynodon dactylon* can also be utilized as a potential therapeutic agent against CHIKV infections [22]. The ethanolic extract of *C. dactylon* was subjected to silica gel column chromatography to obtain anti-CHIKV fraction [22].

Reverse phase-HPLC and GC-MS studies identified the major phytochemicals in the fraction as flavonoids, luteolin, and apigenin as major phytochemicals in the anti-CHIKV ethanolic fraction of *C. dactylon* [22]. Cytotoxicity and the potential of the fraction against CHIKV were evaluated *in-vitro* using Vero cells [22]. The fraction

was found to exhibit potent (about 98%) viral inhibitory activity at very low concentrations, as observed by a reduction in CPE, and the cytotoxic concentration of the fraction was found to be 250 µg/mL [22]. Reduction in viral replication was assessed by reverse transcriptase-polymerase chain reaction (RT-PCR) after treating the viral infected Vero cells with the fraction, which showed that the reduction in viral mRNA synthesis in fraction-treated infected cells was much greater than the viral infected control cells [22].

13. Flavonoids: Flavonoids are a large group of hydroxylated phenolic (polyphenolic) compounds having a benzo-γ-pyrone structure and are synthesized by the phenylpropanoid pathway present in the plant kingdom. Flavonoids are synthesized by plants in response to microbial infections. Generally, the secondary plant metabolites of phenolic nature, including flavonoids, are pharmacologically active. These are also promising antiviral therapeutics against CHIKV *in-vitro* replication. A study showed that three flavonoids - silymarin, quercetin, and kaempferol exhibit significant *in-vitro* antiviral activity against CHIKV using a CHIKV replicon cell line and a clinical isolate of CHIKV of Central/East African genotype [23]. A CPE inhibition assay was performed to determine their inhibitory activities on CHIKV viral replication and a quantitative reverse transcription (RT)-PCR was used to determine virus yield [23]. Antiviral activity of the compounds under study was further investigated by evaluation of CHIKV protein expression using Western Blotting for CHIKV nsP1, nsP3, and E2E1 proteins [23]. Briefly, silymarin reduces CHIKV replication efficiency and down-regulates the production of viral proteins involved in replication [23].

14. Niclosamide and Nitazoxanide: Niclosamide is an orally-bioavailable chlorinated salicylanilide with potential anti-neoplastic and anti-helminthic activity. Nitazoxanide is a broad-spectrum anti-parasitic and antiviral drug that is used for the treatment of various helminthic, protozoal, and viral infections. In one study, a high-throughput screening (HTS) system based on CHIKV 26S mediated insect cell fusion inhibition assay was performed [24]. These are found to exhibit anti-CHIKV-induced CPE, which were further confirmed by RT-qPCR and IFA assays [24]. Moreover, these compounds were observed to limit virus entry, inhibiting both viral release and their cell-to-cell transmission [24]. This study proved that niclosamide and nitazoxanide could inhibit CHIV entry and transmission [24].

15. Piperazine: Piperazine is a small, heterocyclic organic compound that consists of a six-membered ring with two nitrogen atoms at opposite positions in the ring. Piperazine is available as small alkaline deliquescent crystals with a saline taste. Molecular docking studies indicate that piperazine binds to the hydrophobic pocket of CHIKV capsid protein (CP) with high affinity and reduces CHIKV load in Vero cells as indicated by IFA and plaque reduction assay studies [25]. This antiviral property of piperazine opens up avenues for further investigations towards the proper development of piperazine-based anti-CHIKV drugs.

16. Ayurvedic Medicines: Ayurvedic medicine is one of the world's oldest holistic healing systems, which was developed more than 3,000 years ago in India, based on the belief that health and wellness depend on a delicate balance between the mind, body, and spirit of an individual. The botanical extracts of *Tinospora cordifolia, Zingiber officinale, Emblica officinalis,* and *Boswellia serrata* were evaluated in a randomized, double-blind, parallel-efficacy, four-arm, multicentre equivalence drug trial of 24 weeks duration on eligible patients suffering from symptomatic knee osteoarthritis (OA) [26]. It was observed that the mentioned ayurvedic formulations offer a good alternative to glucosamine and celecoxib in the treatment of symptomatic knee OA, which is a common symptom of CHIKV infections [26].

Passive Immunotherapy for CHIKV Infections: Besides the use of new chemicals as anti-CHIKV therapeutics, fortunately enough, passive immunotherapy has proved to offer potential benefits in the treatment of CHIKV infections [27]. Laboratory-based studies on animals receiving passive immunotherapy against CHIKV have yielded promising results [28]. A lipid-encapsulated mRNA encoding a potentially neutralizing human monoclonal antibody gives protection against CHIKV infections [29]. Pre-clinical results showing that passive transfer of neutralizing antibodies can protect humans against CHIKV infections have

paved the pathway for translational clinical trials of mRNA-based passive immunotherapy against the same [30]. Like, passive immunotherapy with CTLA 4-immunoglobulin and an anti-CHIKV monoclonal antibody has been shown to control CHIKV-induced arthritis [31].

Besides, clinical studies using passive immunotherapy in those particularly vulnerable to severe CHIKV infections are currently in progress [31]. Such passive immunotherapy involves the administration of anti-CHIKV hyperimmune human intravenous antibodies (immunoglobulins) to those exposed to a high risk of CHIKV infections [31]. The rapid and sporadic nature of chikungunya outbreaks poses challenges for the planning of large clinical efficacy trials suggesting that licensure of chikungunya vaccines may utilize non-traditional approval pathways based on the identification of immunological endpoint(s) predictive of clinical benefit. This report reviews the current status of nonclinical and clinical testing and potential challenges for defining a suitable surrogate or correlate of protection.

Vaccines against CHIKV Infections: Vaccines are the best strategy to prevent most vector-borne diseases. Ideally, vaccines for diseases in developing countries must combine the attributes of being cheap and of single-dose efficacy. At the same time, a quick and long-lived immune response with a minimum risk of post-vaccine complications should be achieved. Despite the fact that CHIKV resurgence is associated with epidemics of unprecedented magnitude and that over the past decade, a large number of candidate vaccines against CHIKV have been developed, including several of them that have now entered clinical trials, but as of 2017, no clinically-approved vaccines to prevent the outbreak of CHIKV infections are available [32].

Recently, a Phase-II vaccine trial used a live, attenuated CHIKV, with attenuation resulting from only two mutations in the viral E2 glycoprotein [33]. The only CHIKV vaccine that has been tested in humans, namely the strain 181/clone25, is a live-attenuated derivative of Southeast Asian human isolate strain AF15561 [33]. This vaccine was potentially immunogenic in Phase I and Phase II clinical trials, although it induced transient arthralgia as a post-vaccination side-effect in about 8% of the vaccinees [33].

This vaccine subsequently led to the development of resistance towards the virus in 98% of the vaccines tested after 28 days [34]. 85% of the vaccinated individuals retained the resistance even after one year [34]. In one study, the CHIK vaccine strain 181/clone25 (181/25) developed by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) was found to be well-tolerated and highly immunogenic in Phase-I and Phase-II clinical trials, although it induced transient arthralgia in some healthy adult volunteers [35]. The scientists conducted their studies in interferon (IFN)-compromised AG129 mice (defective in IFN-α/β and IFN-γ receptor signaling), and also evaluated the immunogenic potential and protective capacity of CHIK 181/25 [35]. Infection of AG129 mice by CHIK 181/25 resulted in rapid mortality within 3-4 days [35]. In contrast, all infected A129 mice (defective in IFN-α/β receptor signaling) survived with temporary morbidity characterized by ruffled appearance and body weight loss [35]. Infection of A129 mice with CHIK 181/25 induced significant levels of IFN-γ and IL-12 while the inflammatory cytokines, TNFα, and IL-6 remained low [35]. A129 heterozygote mice that retain partial IFN-α/β receptor signaling activity remained healthy [35]. A single administration of the CHIK 181/25 vaccine provided both short-term (38 days post-prime) and long-term protection (247 days post-prime) against challenge with wild-type (WT) CHIKV-La Reunion (CHIKV-LR) [35]. This protection was at least partially mediated by neutralizing antibodies [35]. Overall, these experimental results highlighted the importance of IFNs in controlling CHIK 181/25 vaccine, and demonstrate the ability of this vaccine to elicit antibody responses that confer short-and long-term protection against WT CHIKV-LR infections [35].

In another study, a Vero cell-adapted, purified, formalin-inactivated prototype whole virus vaccine candidate was prepared using a current Indian strain (the ECSA genotype) implicated with the explosive CHIK fever epidemic during 2006 [36]. The assessment of both humoral and cell-mediated immune responses in mice injected with this vaccine was accomplished through ELISA, plaque

reduction neutralization test (PRNT), micro-cytotoxicity assay and cytokine production assay [36].

All these experimental findings suggested that this formalin-inactivated CHIKV vaccine candidate reported in the study had the excellent immunogenic potential to neutralize the CHIKV infectivity by enhancing both humoral and cell-mediated immune responses [36]. Levitt and colleagues in 1986 developed the first live attenuated vaccine strain for CHIKV meant for human use [37]. The vaccine (pilot-lot production) was able to replicate in both species of the vector *A. aegepti* and *A. albopictus*, and was able to elicit neutralizing antibodies to protect mice and rhesus monkeys against CHIKV challenge [37]. This was proved by the absence of viremias in vaccinated monkeys [37]. This vaccine was then produced and tested in accordance with government rules and regulations [37]. Yeast-derived Chikungunya Virus-Like Particles (CHIK-VLPs) have been expressed by *Pichia pastoris*, and evaluated for neutralizing activity against CHIKV [38]. Neonatal mice receiving anti-CHIK-VLPs antibodies were protected from CHIKV challenge through induction of cellular immune response by higher levels of TNF-α and IL-10, and substantial levels of IL-2, IL-4, and IFN-γ [38]. A rational attenuation mechanism was formulated that also prevents the infection of mosquito vectors by CHIKV [38]. The internal ribosome entry site (IRES) from encephalo-myocarditis virus was made to replace the subgenomic promoter in a cDNA CHIKV clone, hence changing the levels and host-specific mechanisms of gene expression of structural proteins [38]. Studies in murine models indicated that the new vaccine was attenuated, but highly immunogenic even after a single administration [38].

In another study, the immunogenicity profile and the efficacy of a novel, viral envelope-based synthetic anti-CHIKV DNA vaccine candidate based on the highly attenuated Poxvirus vector modified Vaccinia virus Ankara (MVA), expressing all the structural genes of CHIKV (capsid/C, E3, E2, 6K, and E1) and called MVA-CHIKV, was studied [39]. It was found that MVA-CHIKV elicited robust innate immune responses in human macrophages and monocyte-derived dendritic cells, with the production of IFN-β, chemokines, and pro-inflammatory cytokines [39]. This potential vaccine candidate induced strong, broad, highly poly-functional, and long-lasting humoral-(neutralizing antibodies against CHIKV), as well as cell-mediated (CHIKV-specific $CD8^+$ T cell responses)-immune responses in rhesus macaques [39]. Remarkably, a single dose of MVA-CHIKV gave protection in the experimental animals after a high-dose challenge with CHIKV [39].

All these results strongly support the potential application of MVA-CHIKV as a vaccine candidate against CHIKV. These new vaccination strategies that can reduce the toll of death and neurological sequelae resulting from infections with CHIKV, once validated by successful human trials, would hopefully open up novel avenues for the treatment and prevention of CHIKV infections in the future. However, there are some challenges before the development of anti-CHIKV vaccines. One of the biggest challenges is that numerous different virus strains are used in the different experiments, along with using different animal models, different routes of both vaccination and challenge, and different methods for evaluating the efficacy of the proposed vaccines. Another challenge is to develop vaccines for a wide range of users. As different approaches may be needed for separate groups, a single approach can be highly difficult to be screened out for a targeted application.

Role of Homoeopathic Medicines in Alleviating the Pathogenicity and Symptoms of CHIKV Infections: Alternative and complementary medicines, including homeopathy, are used by many patients for the conventional treatment of diseases [40]. Various attempts have been made to find out the prospects of homeopathic drugs to treat CHIKV disease-associated inflammations. Experiments have shown that some homeopathic medicines such as *Bryonia alba* (Bryonia), *Rhus toxicodendron* (Rhus tox), and *Polyporus pinicola* (Pine agaric) have inhibitory effects against CHIKV [40]. *Toxicodendron pubescens* P. Mill (Anacardiaceae), known in homeopathy as *Rhus toxicodendron* (Rhus tox), is widely used as an anti-inflammatory homeopathic medicine [40]. *Rhus tox* in homeopathic dilution appeared to interfere with inflammatory processes involving the physiologically-active mediators like histamine, prostaglandins, leukotrienes, and others [40].

Initial experimentations were carried out with different *Rhus tox* concentrations, of which one was found to significantly reduce carrageenan-induced paw oedema and vascular permeability in Wistar rats and mice, besides alleviating writhing induced by intraperitoneal acetic acid and gastric lesions induced by stress [40]. These results were found to be similar to those obtained in another set of murine-model-based experiments carried out using *Rhus tox* 200C (a homeopathic dilution) treatments on rats and studying the changes in their behavioral patterns [41].

In another study, *Rhus tox* in both its crude form and homeopathic dilutions (6C, 30C, 200C) was assessed for reducing the symptoms of Complete Freund's Adjuvant (CFA)-induced arthritis in rats [42]. The severity of arthritis in the animal models was followed through observation of changes in inflammatory lesions, body and organ weight, and hemato-immunological parameters, including C-reactive protein (CRP) [42]. It was found that *Rhus tox* offered protection to rats from CFA-induced inflammatory lesions, body, and organ weight changes, and hemato-immunological alterations [42]. All the homeopathic dilutions of *Rhus tox*, as well as the crude form, showed anti-arthritic activity [42].

This study thus supported claims in the homeopathic literature on the role of *Rhus tox*, and its ultra dilutions in the treatment of arthritis and associated pain. A study in Kerala, India, revealed that all the systems of medicine (allopathy, Ayurveda, homeopathy, or traditional) are equally important for the management of chikungunya [43]. This study conducted on 1061 people living in different parts of Kerala, most affected by CHIKV epidemic, showed that the homeopathic medicine *Eupatorium perfoliatum* 200C helped prevent chikungunya in 82.19% of the cases [43]. It was observed that *Bryonia alba* 30C as genus epidemicus was better than placebo in decreasing the incidence of CHIKV virus in Kerala [43]. Nair *et al*., studied the effect of homeopathic therapy in 126 patients, some with Chikungunya fever (CF) and some with Post-Chikungunya Chronic Arthritis (PCCA) in a primary health care setting named Delhi Government Homeopathic Dispensary, Aali Village, New Delhi, India [44]. Complete recovery was observed in 84.5% CF cases in a mean time of 6.8 days, whereas 90% cases of PCCA were found to recover completely in a mean time of 32.5 days post-treatment [44]. Hence it was proposed that homeopathic therapy may prove to be highly effective in CF and PCCA [44].

CONCLUSION: Because CHIKV infections were of a magnum magnitude and the CHIKV has left a devastating effect in the last decade, an appreciable effort has been devoted to assessing, designing, and developing products for both prophylaxis and treatment of the CHIKV infections. However, like most arthropod-borne viral outbreaks, as the pandemics subside, the drive to develop potential antivirals against CHIKV tends to fade over time. But owing to the fatal and enfeebling nature of the disease and an increasingly large number of individuals who are prone to contract CHIKV infections, assessment of novel therapeutics and vaccine development against CHIKV infections are immediately required and needed to be continued far. Although the challenges in developing a CHIKV vaccine are numerous, the science behind all of the approaches described in this article is fairly sound, and given the great transmissibility and virulence of CHIKV, the development of both vaccines and new therapeutics for distribution is an aim worth to achieve.

Additional effort should be devoted in promoting confirmation to the basic guidelines and maintaining proper documentation regarding the prevention and treatment of CHIKV infections, which will definitely help to achieve the goal of curbing CHIKV and the associated disease. It is an absolute mandate now that we expand and extend our effort and promote this awareness to the very grass-root level, which will not only offer the health-care sector officials with new ideas in pharmaceutical technology directed against CHIKV infections but, in turn, will also help benefit our society and community as a whole.

ACKNOWLEDGEMENT: The authors are deeply grateful to the authorities of St. Xavier's College (Autonomous), Kolkata and Peerless Hospital & B. K. Roy Research Centre, Kolkata for their constant inspiration and motivation.

CONFLICTS OF INTEREST: The authors declare that there is no potential conflict of interest between them.

REFERENCES:

1. Ray D and Shi PY: Recent advances in flavivirus antiviral drug discovery and vaccine development. Recent Patents on Anti-Infective Drug Discovery 2006; 1(1): 45-55.
2. Staples JE and Breiman RF: Chikungunya fever: an epidemiological review of a re-emerging infectious disease. Clinical Infectious Diseases 2009; 49(6): 942-48.
3. Couderc T, Chrétien F, Schilte C, Disson O, Brigitte M, Guivel-Benhassine F and Touret Y: A mouse model for Chikungunya: young age and inefficient type-1 interferon signaling are risk factors for severe disease. PLoS Pathogens 2008; 4(2): e29.
4. Ganesan VK, Duan B and Reid SP: Chikungunya Virus: Pathophysiology, Mechanism, and Modeling. Viruses 2017; 9: 368-82.
5. Burt FJ, Chen W, Miner JJ, Lenschow DJ, Merits A, Schnettler E and Kohl A: Chikungunya virus: an update on the biology and pathogenesis of this emerging pathogen. The Lancet Infectious Diseases 2017; 17(4): e107-e117.
6. Morrison TE, Oko L, Montgomery SA, Whitmore AC, Lotstein AR, Gunn BM and Elmore SA: A mouse model of chikungunya virus induced musculoskeletal inflammatory disease. The American Journal of Pathology 2011; 178(1): 32-40.
7. Edwards CJ, Welch SR and Chamberlain J: Molecular diagnosis and analysis of Chikungunya virus. Journal of Clinical Virology 2007; 39(4): 271-75.
8. Johnson BW, Goodman CH, Holloway K, Martinez de Salazar P, Valadere AM and Drebot MA: Evaluation of commercially available chikungunya virus immune-globulin M detection assays. The American Society of Tropical Medicine and Hygiene 2016; 95(1): 182-92.
9. Lani R, Hassandarvish P, Shu MH, Hong Phoon W, Hann Chu JJ, Higgs S and Vanlandingham D: Antiviral activity of selected flavonoids against Chikungunya virus. Antiviral Research 2016; 133: 50-61.
10. Erasmus JH, Needham J, Raychaudhuri S, Diamond MS, Beasley DWC, Morkowski S and Salje H: Utilization of an eilat virus-based chimera for serological detection of chikungunya infection. PLoS Neglected Tropical Diseases 2015; 9(10): e0004119.
11. Singh P, Chhabra M, Mittal V, Sharma P, Rizvi MA, Chauhan LS and Rai A: Current research and clinical trials for a vaccine against Chikungunya virus. Vaccine: Development and Therapy 2013; 3: 35-46.
12. Gallegos KM, Drusano GL, D′Argenio DZ and Brown AN: Chikungunya virus: *in-vitro* response to combination therapy with ribavirin and interferon Alfa 2a. The Journal of Infectious Diseases 2016; 214(8): 1192-97.
13. Feibelman KM, Fuller BP, Li L, LaBarbera DV and Geiss BJ: Identification of small molecule inhibitors of the Chikungunya virus nsP1 RNA capping enzyme. Antiviral Research 2018; 154: 124-31.
14. Gigante A, Gómez-SanJuan A, Delang L, Li C, Bueno O, Gamo AM and Priego EM: Antiviral activity of [1,2,3] triazolo [4,5-d]pyrimidin-7(6H)-ones against chikungunya virus targeting the viral capping nsP1. Antiviral Research 2017; 144: 216-22.
15. Dhindwal S, Kesari P, Singh H, Kumar P and Tomar S: Conformer and pharmacophore based identification of peptidomimetic inhibitors of chikungunya virus nsP2 protease. J of Bio Str and Dyn 2017; 35(16): 3522-39.
16. Kuo SC, Wang YM, Ho YJ, Chang TY, Lai ZZ, Tsui PY and Wu TY: Suramin treatment reduces chikungunya pathogenesis in mice. Antiviral Research 2016; 134: 89-96.
17. Sharma R, Fatma B, Saha A, Bajpai S, Sistla S, Dash PK and Parida M: Inhibition of chikungunya virus by picolinate that targets viral capsid protein. Virology 2016; 498: 265-76.
18. Delogu I, Pastorino B, Baronti C, Nougairède A, Bonnet E and Lamballerie X: *In-vitro* antiviral activity of Arbidol against Chikungunya virus and characteristics of a selected resistant mutant. Antiviral Research 2011; 90(3): 99-107.
19. Varghese FS, Kaukinen P, Glasker S, Bespalov M, Hanski L, Wennerberg K and Kümmerer BM: Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses. Antiviral Research 2016; 126: 117-24.
20. Varghese FS, Thaa B, Amrun SN, Simarmata D, Rausalu K, Nyman TA and Merits A: The Antiviral Alkaloid Berberine Reduces Chikungunya Virus-Induced Mitogen-Activated Protein Kinase Signaling. Journal of Virology 2016; 90 (21): 9743-57.
21. Mounce BC, Cesaro T, Carrau L, Vallet T and Vignuzzi M: Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. Antiviral Research 2017; 142: 148-157.
22. Murali KS, Sivasubramanian S, Vincent S, Murugan SB, Giridaran B, Dinesh S and Gunasekaran P: Anti-chikungunya activity of luteolin and apigenin rich fraction from Cynodondactylon. Asian Pacific Journal of Tropical Medicine 2015; 8(5): 352-58.
23. Lani R, Hassandarvish P, Chiam CW, Moghaddam E, Chu JJ, Rausalu K and Merits A: Antiviral activity of silymarin against chikungunya virus. Scientific Reports 2015; 5: 11421.
24. Wang YM, Lu JW, Lin CC, Chin YF, Wu TY, Lin LI and Lai ZZ: Antiviral activities of niclosamide and nitazoxanide against chikungunya virus entry and transmission. Antiviral Research 2016; 135: 81-90.
25. Aggarwal M, Kaur R, Saha A, Mudgal R, Yadav R, Dash PK and Parida M: Evaluation of antiviral activity of piperazine against Chikungunya virus targeting hydrophobic pocket of alphavirus capsid protein. Antiviral Research 2017; 146: 102-11.
26. Chopra A, Saluja M, Tillu G, Sarmukkaddam S, Venugopalan A, Narsimulu G and Handa R: Ayurvedic medicine offers a good alternative to glucosamine and celecoxib in the treatment of symptomatic knee osteoarthritis: a randomized, double-blind, controlled equivalence drug trial. Rheumatology 2013; 52(8): 1408-17.
27. Chu H, Das SC, Fuchs JF, Suresh M, Weaver SC, Stinchcomb DT and Partidos CD: Deciphering the protective role of adaptive immunity to CHIKV/IRES a novel candidate vaccine against Chikungunya in the A129 mouse model. Vaccine 2013; 31(33): 3353-60.
28. Smalley C, Erasmus JH, Chesson CB and Beasley DWC: Status of research and development of vaccines for chikungunya. Vaccine 2016; 34(26): 2976-81.
29. Kose N, Fox JM, Sapparapu G, Bombardi R, Tennekoon RN, de Silva AD and Elbashir SM: A lipid-encapsulated mRNA encoding a potently neutralizing human monoclonal antibody protects against chikungunya infection. Science Immunology 2019; 4(35): eaaw6647.
30. Williams I: mRNA-based passive immunotherapy. Science 2019; 364(6442): 746-748.
31. Miner JJ, Cook LE, Hong JP, Smith AM, Richner JM, Shimak RM and Young AR: Immunotherapy with CTLA4-Ig and an antiviral monoclonal antibody controls chikungunya virus arthritis. Science Translational Medicine 2017; 99375: eaah3438.

32. Milligan GN, Schnierle BS, McAuley AJ and Beasley DWC: Defining a correlate of protection for chikungunya virus vaccines. Vaccine 2019; 37(50): 7427-36.
33. Gorchakov R, Wang E, Leal G, Forrester NL, Plante K, Rossi SL and Partidos CD: Attenuation of Chikungunya virus vaccine strain 181/clone 25 is determined by two amino acid substitutions in the E2 envelope glycoprotein. Journal of Virology 2012; 86(11): 6084-96.
34. Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG and Mangiafico JA: Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. The American Journal of Tropical Medicine and Hygiene 2000; 62(6): 681-85.
35. Partidos CD, Weger J, Brewoo J, Seymour R, Borland EM, Ledermann JP and Powers AM: Probing the attenuation and protective efficacy of a candidate chikungunya virus vaccine in mice with compromised interferon (IFN) signaling. Vaccine 2011; 29(16): 3067-73.
36. Tiwari M, Parida M, Santhosh SR, Khan M, Dash PK and Rao PV: Assessment of immunogenic potential of Vero adapted formalin inactivated vaccine derived from novel ECSA genotype of Chikungunya virus. Vaccine 2009; 27(18): 2513-22.
37. Tharmarajah K, Mahalingam S and Zaid A: Chikungunya: vaccines and therapeutics. F1000Res 2017; 6: 2114.
38. Saraswat S, Athmaram TN, Parida M, Agarwal A, Saha A and Dash PK: Expression and characterization of yeast derived chikungunya virus like particles (CHIK-VLPs) and its evaluation as a potential vaccine candidate. PLoS Negl Trop Dis 2016; 10(7): e0004782.
39. Garcia-Arriaza J, Cepeda V and Hallengard D: A novel poxvirus-based vaccine (MVA-CHIKV) is highly immunogenic and protects mice against chikungunya infection. Journal of Virology 2014; 88(6): 3527-47.
40. dos Santos AL, Perazzo FF, Cardoso LG and Carvalho JC: *In-vivo* study of the anti-inflammatory effect of *Rhus toxicodendron*. Homeopathy 2007; 96(2): 95-101.
41. Rocha da Silva MP, Soares FM, Martini LC and Bonamin L: Behavior of rats treated with *Rhus toxicodendron* 200cH. International Journal of High Dilution Research 2008; 7(22): 3-6.
42. Patil CR and Rambhade AD: Modulation of arthritis in rats by *Toxicodendron pubescens* and is homeopathic dilutions. Homeopathy 2011; 100(3): 131-37.
43. Dilip C, Saraswathi R, Krishnan PN, Azeem AK, Raseena, Azeez A and Ramya: Comparative evaluation of different systems of medicines and the present scenario of Chikungunya in Kerala. Asian Pacific Journal of Tropical Medicine 2010; 3(6): 443-47.
44. Wadhwani GG: Homeopathic drug therapy. homeopathy in chikungunya fever and post-chikungunya chronic arthritis: an observational study. Homeopathy 2013; 102(3): 193-98.

How to cite this article:
Roy S, Bhattacharjee A, Roy L and Das S: Recent advances towards the development and applications of novel therapeutics against chikungunya virus infections. Int J Pharm Sci & Res 2020; 11(12): 6503-13. doi: 10.13040/IJPSR.0975-8232.11(12).6503-13.