CLINICAL INVESTIGATION

The challenges of dengue drug discovery and development

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Keywords: antiviral • dengue • drug discovery • flavivirus

The four serotypes of dengue virus (DENV) represent the most prevalent mosquito-borne viral pathogen. DENV is endemic in all tropical and subtropical areas in the world, with approximately 390 million human infections annually, of which 96 million infections manifest symptoms [1]. DENV infection could lead to dengue fever, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). The disease outcome is controlled by both viral and host factors. Immune response to DENV infection, such as cytokine production, could contribute to increased vascular permeability, leading to severe DHF/DSS. Currently, there is no clinically approved anti-DENV therapy or vaccine. Lifelong immunity following infection is only protective to the same serotype; a secondary infection by a different serotype may result in more severe symptoms [2]. Therefore, a successful vaccine requires a balanced immunity against all four serotypes of DENV. Such challenges of dengue vaccine underscores the urgency for antiviral development. This editorial aims to summarize the current status of dengue drug discovery, focusing on compounds that have been tested in dengue clinical trials, and highlighting the challenges for dengue drug discovery.

The ideal treatment for dengue is a safe, pan-serotype active, fast-acting direct antiviral with high resistance barrier. Nucleoside and nucleotide inhibitors of polymerase fulfill these criteria. Balapiravir was the first nucleoside analog and the only direct antiviral inhibitor that has entered clinical trial in dengue patients [3]. Balapiravir is a prodrug of 4′-azidocytidine that was initially developed for hepatitis C virus (HCV). The compound was terminated owing to toxicity after a long-term treatment required for HCV therapy [4]. Since Balapiravir is active against DENV in cell culture, it was repurposed for potential dengue therapy. Adult dengue patients within 48 h of onset of fever were given 1500 mg (n = 10) or 3000 mg (n = 22) twice daily for 5 days. Although the drug was well tolerated, viremia and fever clearance time as well as plasma cytokine profile showed no difference to that of patients receiving placebo (n = 32). Two possible reasons have been proposed for the lack of efficacy in dengue patients [5]: Balapiravir decreased its potency when treating human peripheral blood mononuclear cells (one of the DENV replication sites in patients) that were preinfected with DENV; and the compound exhibited weak antiviral activities in different nonhematopoietic cells. Both reasons stem from the requirement of host kinases to convert nucleoside analog to its triphosphate form – the active ingredient for antiviral activity.

Besides Balapiravir as a viral target inhibitor, a number of host inhibitors that suppress DENV replication and/or modulate host immune response have been tested in clinical trials. Chloroquine has long been used as an antimalarial drug. It is a weak base and could exert anti-DENV activity by interfering with the acidic pH of endolysosomal compartments and the *trans*-Golgi network in which the virus undergoes fusion and maturation. Chloroquine was dosed according to the WHO recommended treatment regimen in 153 dengue patients. The outcome of the trial was negative, with no difference in fever

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duration between the chloroquine-treated and placebo patients [6]. The host immuopathogenesis has long been the center of dengue research and is believed to be the main cause leading to severe DHF/DSS. A broad anti-inflammatory drug is postulated to be able to block the cytokine release, reduce vascular damage and prevent DHF/DSS. Therefore, prenisolone, a highly effective and safe anti-inflammatory agent, was given to 150 patients in acute phase of DENV infection (≤72 h after onset of illness). Unfortunately, the treatment did not result in reduction in the development of shock nor other severe symptoms evaluated by a wide range of clinical and virological end points [7]. Celgosivir (6-*O*-butanoyl castanospermine) is an ester prodrug of castanospermine, an inhibitor of cellular α-glucosidase I and II. The host α-glucosidase is required for carbohydrate modification of DENV glycoproteins, such as prM, E and NS1. The compound inhibits all four serotypes of DENV in cell culture as well as in a mouse efficacy model [8,9]. Besides DENV, Celgosivir and castanospermine also inhibit HIV-1, HCV, influenza virus and many other viruses [10]. Celgosivir was previously tested in HCV patients, but was terminated owing to side effects [10]. Since HCV needs much longer treatment than DENV in patients, it was hoped that the short treatment in dengue patients would mitigate the side effects of Celgosivir. Therefore, Celgosivir was repurposed for dengue therapy. Although well tolerated, Celgosivir did not reduce viremia nor fever burden in dengue patients [11]. Similar to Celgosivir, UV-4, a derivative of the deoxynojirimycin and also an inhibitor of cellular α-glucosidase, has anti-DENV activity in cell culture and mouse lethal model [12]. A Phase I trial is currently ongoing to assess safety, tolerability and pharmacokinetics of UV-4 in healthy volunteers. Cholesterol is required for DENV replication in cell culture [13]. Therefore, lovastatin, a cholesterol pathway inhibitor, has the potential to be repurposed for dengue therapy. The pleiotropic beneficial effects on endothelial function and reduction of inflammation of statin is also expected to prevent DHF/DSS. A lovastatin trial is currently ongoing to assess the safety and efficacy in dengue patients [14]. A recent study showed that treatment with lovastatin increased survival of DENV-infected mice; however, an increased viremia was observed in the infected mice when lovastatin was administered after virus infection [15].

Three approaches have been pursued for preclinical drug discovery [16]: inhibitors of viral proteins, including proteins with enzymatic activities (e.g., NS3 protease and NS5 polymerase), proteins without enzymatic activities (e.g., NS4B), and proteins required for viral entry and virion assembly (e.g., capsid and envelope); inhibitors of host proteins that are required for viral replication (e.g., cellular α-glucosidase); and inhibitors that block the pathological pathways that lead to dengue diseases (e.g., prednisolone). A recent review has summarized the current status of these efforts [17]. For each of the above approaches, obstacles have been encountered. For inhibitors of viral targets, identifying a compound that is active against all four serotypes of DENV is challenging. This is due to the variation of amino acid sequence of approximately 30–35% among the four DENV serotypes [18], similar to the level of variation among the seven HCV genotypes (which has been proven challenging for identification of pan-genotype inhibitors). For inhibitors of host targets, identifying a compound that can selectively inhibit viral replication without compromising the normal function of host protein is challenging. For targeting disease-causing pathological pathways, the challenge is the lack of knowledge at a molecular level of the pathway so that specific host factors could be screened for pharmacological inhibition.

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Besides the small molecule approach, therapeutic antibodies have been explored for dengue therapy [19,20]. The challenges of antibody approach include that at least four antibodies are needed to inhibit the four serotypes, the intravenous dosing (instead of oral dosing for small molecule inhibitors), high cost of goods and potential low barrier of resistance emergence [21]. These challenges will limit the use of the antibody therapy in patients living in underdeveloped regions.

Compared with treatments with chronic viral infections (e.g., HIV and HCV), the treatment duration of acute DENV infection is expected to be less than a week. Therefore, the DENV inhibitors need to be fast acting, and the infected patients should be treated as early as possible. For each class of inhibitors, emergence of resistant DENV should be characterized under laboratory and clinical settings. To overcome potential resistance, a combination of two antivirals with distinct mode of action will probably be needed to minimize resistance and/or to ensure inhibition on all serotypes. The combination can be from two direct antivirals or between a direct antiviral and a host antiviral inhibitor.

To conclude, several clinical trials in dengue patients have been carried out in recent years. Although none has shown any efficacy so far, more research and more trials are ongoing. It is notable that all the compounds so far tested in dengue clinical trials are repurposed

from molecules developed for other indications. *Bona fide* inhibitors that are specifically designed for DENV remain to be developed. With more academic laboratories and pharmaceutical companies joining the fight against dengue, there is strong hope that we will have safe and efficacious anti-dengue drugs in the foreseeable future. Indeed, testing direct-acting anti-DENV drugs in patient trials is the only way to determine whether the antiviral approach is of therapeutic value.

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