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SUPPLEMENT ARTICLE

Small Molecules and Antibodies for Zika Therapy

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Zika virus (ZIKV) infection during pregnancy can cause devastating congenital abnormities or fetal demise. Zika virus infection could also cause Guillain-Barré syndrome in adults. Mosquito control, vaccine, and therapeutics are 3 potential, effective means to prevent ZIKV infection. Here we review the current status of ZIKV drug discovery. Both small molecule inhibitors and therapeutic antibodies have been identified, some of which have shown promising efficacy in mouse models. Most inhibitors were identified through screening US Food and Drug Administration–approved drugs and clinical trial compounds; however, none of them were potent enough to justify a ZIKV clinical trial. Such a repurposing approach has also been pursued for dengue therapy, with several compounds tested in clinical trials showing no clinical benefits. Because pregnant women are the main target population for ZIKV treatment, therapeutic candidates could be developed through a 2-stage path. The first stage should demonstrate safety and efficacy in nonpregnant patients. Once efficacy has been demonstrated in nonpregnant patients, the candidates should be rapidly advanced to stage 2 for safety and efficacy evaluation in pregnant patients. The 2-stage developmental path is supported by previous results from trials with other viral infections that showed that treatment of pregnant women with antiviral drugs or hyperimmunoglobulins significantly reduced congenital abnormalities in neonates.

Keywords. Zika; therapeutics; drug discovery.

Many flaviviruses are important human pathogens that are transmitted by mosquitoes or ticks, including yellow fever, dengue, Japanese encephalitis, West Nile, Zika, and tick-borne encephalitis viruses. In the past few years, Zika virus (ZIKV) has explosively emerged as a major public health threat. Zika virus is primarily transmitted by *Aedes spp*. mosquitoes. It can also infect humans through sexual route, blood transfusion, organ transplantation, and maternal–fetal transmission [1–10]. Therefore, mosquito control represents an effective means to control ZIKV and other arbovirus transmissions. Besides vector control, vaccine and therapeutics are 2 other potential countermeasures to prevent and treat ZIKV infection. It is conceivable that once a vaccine becomes available, immunization of the general population living in endemic areas and prophylactic immunization of travelers would effectively prevent ZIKV infection. As a complementary means, therapeutics could be used to treat infected individuals or travelers to high-risk epidemic regions. In the past 2 years, vaccine development, including development of inactivated, subunit (prM-E proteins expressed from DNA, RNA, or viral vectors), and live-attenuated vaccines [11–20], has progressed at an unprecedented pace. Several ZIKV vaccine candidates have already entered phase I clinical trials [16, 21]. Compared with vaccine development, development of ZIKV therapeutics has been lagging. A number of laboratories have identified small molecular inhibitors and therapeutic antibodies, some of which have shown in vitro and in vivo efficacy; however, none of them has entered clinical development.

GENERAL ANTIVIRAL APPROACHES

Four general antiviral approaches have been pursued for therapeutic development. The first is inhibition of the viral target. Many antiviral drugs currently in clinical use are inhibitors of viral proteins, such as human immunodeficiency virus (HIV) reverse-transcriptase, protease, and integrase. This approach has contributed to almost all of the antiviral drugs currently in clinical use. The second is inhibition of the host factor that is required for viral infection. This approach has led to the development of Maraviroc, a clinically approved HIV drug that inhibits host protein CCR5, a coreceptor for HIV entry [22]. The third is immune modulation. Interferon, a potent cytokine that triggers protective defenses of the immune system to eradicate pathogens, has been clinically used for treatment of hepatitis C and other viral infections [23, 24]. Besides interferon, small molecules targeting different components of the innate immune response (such as RIG-1, MDA5, and STING), either agonist or antagonist, are being pursued for potential treatment of infectious diseases and other indications [25, 26]. The fourth is inhibition of the disease pathway. Because disease pathology is often caused by overreaction of the immune response to viral infection (rather than by direct viral infection), development of molecules that can pharmacologically modulate the pathway of disease development is conceivable. This approach is particularly attractive for treatment of acute viral infections because

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these infections often exhibit a short duration of viremia that declines to an undetectable level within a few days. However, such an approach requires a clearly defined molecular mechanism of the disease pathway, which is currently missing for many viral infections. All 4 of the above approaches could potentially be considered for ZIKV therapeutics development.

DISEASE INDICATIONS FOR TREATMENT

For any drug development, it is critical to identify specific disease symptoms that need to be pharmacologically treated and prevented in patients. In the case of ZIKV, approximately 20% of infected individuals are estimated to develop clinical symptoms; however, a recent retrospective serosurvey in French Polynesia showed that approximately 70% of seropositive infants and 50% of seropositive adults were symptomatic [27]. Among the various disease symptoms, 2 indications should be particularly targeted for treatment: congenital Zika syndrome (CZS) in developing fetuses or infants and Guillain-Barré syndrome (GBS) in adults. Congential Zika syndrome represents the most devastating manifestation of ZIKV infection; it consists of a wide array of congenital abnormalities in infants, including microcephaly, craniofacial disproportion, spasticity, seizures, ocular abnormalities, cerebral calcification, and miscarriage [28, 29]. Guillain-Barré syndrome is an autoimmune neurological disease characterized by ascending paralysis in some adults after ZIKV infection [30]. The molecular mechanism of ZIKV-mediated GBS is not well understood; no in vitro and in vivo systems have been developed to model this disease. These limitations make it challenging to launch a productive drug discovery effort against GBS at this time. Therefore, development of an experimental system that can recapitulate GBS will be the prerequisite for any therapeutic projects on this indication.

Besides CZS and GBS, ZIKV therapeutics should also prevent viral replication and transmission. Compared with other flaviviruses, 1 unique feature of ZIKV is its ability to persist in the male reproductive tract, as infectious virus and viral RNA have been detected in semen up to 69 and 188 days after symptom onset, respectively [31–33]. Infected males can transmit the virus to sexual partners during the period of persistent infection [34]. Therefore, an antiviral agent should be able to access the persistent replication site(s) and eliminate viral replication and sexual transmission.

EXPERIMENTAL SYSTEMS FOR DRUG DISCOVERY

Various experimental systems have been established to enable different approaches for drug discovery. The first system involves the structures of virion and viral proteins. Enormous structural information has been obtained to allow structure-based drug discovery, including the cryo-EM structure of ZIKV [35, 36] and the crystal structures of ZIKA envelope [37], NS1 [38, 39], NS3 proteinase and helicase [40–42], and NS5 methyltransferase and polymerase [43–45]. The second system involves reverse genetic systems. Infectious cDNA clones have been developed for various strains of ZIKV to facilitate antiviral discovery [46–51]. The reverse genetic systems could be used to engineer resistance mutations for target deconvolution of inhibitors. Reporter ZIKV and replicons have also been established for high-throughput screening of large compound libraries [46, 52]. The expression level of reporter genes, such as luciferase or mCherry, could be used as a surrogate to measure viral replication level and inhibitory activity of drugs or antibodies [53, 54]. The third system involves animal models. Various rodent models have been reported to study viral pathogenesis [55, 56], sexual transmission [57, 58], maternal-fetal transmission, and male reproductive tract injury [59, 60]. In addition, nonhuman primate models have been developed to investigate pathogenesis and sexual transmission [61–63]. These models could be used to test the in vivo efficacy of therapeutic agents.

SMALL MOLECULE INHIBITORS

Both small molecule inhibitors and therapeutic antibodies were reported to have anti-ZIKV activity in cell culture, and some have shown efficacy in mouse models. An ideal small molecule drug would be orally bioavailable with good efficacy and an extensive systemic distribution (because ZIKV seems to infect various tissues and organs [64–66]). Because treatment of pregnant women to prevent CZS is the major goal, the drug must potently inhibit ZIKV replication without toxicity across all relevant cell types, such as dendritic, epithelial, fibroblast, liver, neuronal, placental trophoblast, and Hofbauer cells. The safety profile of the compounds should qualify for pregnancy category A/B drugs that do not impair the growing fetus or the maternal–fetal interface [67]. Because the immune system is critical for prevention of maternal–fetal cross-transmission [68–71], the compounds should not interfere with the normal function of the antiviral immune response.

Two major categories of small molecule inhibitors have been reported for ZIKV: nucleoside/nucleotide and nonnucleoside inhibitors. Within the nucleoside/nucleotide category, MK-0608 (an adenosine analog), BCX4430 (an adenosine analog), NITD008 (an adenosine analog), and sofosbuvir (a uridine prodrug) exhibited modest in vitro and in vivo efficacy [72–74]. Among these, sofosbuvir is a clinically approved drug for hepatitis V virus (HCV) treatment. None of the nucleoside/nucleotide compounds were able to completely prevent ZIKV-infected mice from death. In the case of sofosbuvir, it should be noted that the in vitro efficacies against ZIKV and HCV are dramatically different, with EC_{50} (compound concentration required to inhibit 50% of virus production) values of 1.4 μ M [75] and 30 nM [76] in Huh7 cells, respectively; the 47-fold difference in EC_{50} values may explain the low in vivo efficacy of sofosbuvir against ZIKV; furthermore, sofosbuvir was pharmacologically

designed to specifically load the active compound in liver where HCV replicates. Because nucleosides/nucleotides represent the cornerstone of antiviral drugs [77], it is well justified to continue the search for ZIKV inhibitors within this category of chemical space. One advantage of nucleoside/nucleotide inhibitors is the broad antiviral spectrum against closely related viruses due to the conserved target sites of viral polymerases. Indeed, 2 of the above 4 nucleoside inhibitors—MK0608 and NITD008—have potent anti–dengue virus (DENV) activities [78, 79].

Inhibitors from the nonnucleoside category were primarily identified through cell-based screenings using a ZIKV infection assay. The screening effort has focused primarily on US Food and Drug Adminstration (FDA)–approved drugs, clinical trial drug candidates, and pharmacologically active compounds. Such repurposing efforts have identified the lipopeptide antibiotic daptomycin, the antimalaria drug mefloquine, the nausea/vomiting drug palonosetron, the immune-suppressant drug mycophenolate mofetil, the pan-caspase inhibitor emricasan, the antiparasitic drugs niclosamide and ivermectin, antimalarial compounds, and the pyrimidine synthesis inhibitor brequinar, among which daptomycin, palonosetron, and niclosamide belong to pregnancy category B drugs [80–83]. Unfortunately, none of these compounds have shown any in vivo activity, most likely because of their low in vitro efficacy. Among the identified compounds, pyrimidine synthesis inhibitors have been well documented to have pan-antiviral in vitro activity but no in vivo efficacy, mainly because the savage pathway could restore pyrimidine concentration in animals (even when de novo synthesis of pyrimidine has been potently suppressed by compounds like brequinar) [84]. The pan-caspase inhibitor emricasan (a phase II compound under investigation for fatty acid liver diseases) was identified because the screen assay used ZIKV infection–induced caspase-3 activity as the assay readout; further analysis showed that emricasan did not directly inhibit viral replication, but rather prevented the infected cells from apoptosis. One potential use of emricasan is to combine it with a direct antiviral inhibitor so the combination therapy can prevent infected cells from death as well as block viral replication [83].

ISSUES FOR THE REPURPOSING APPROACH

Although screening of FDA-approved drugs is an attractive approach to identify clinically approved compounds for new indications, there are 2 key criteria that must be met before further development. First, the exposure level of drug in humans should exceed the efficacious concentration determined in cell culture (eg, EC_{90} values). For clinically used drugs, human pharmacological data are usually available for such assessment. Second, because different diseases are often associated with different tissues or organs, it is important to focus on the drug exposure levels in the disease-related tissues or organs. Currently, no repurposed drugs have entered clinical trials for ZIKV.

A number of lessons could be learned from the repurposing effort for DENV. So far, 5 repurposed compounds have been completed in clinical trials for DENV treatment: chloroquine (an inhibitor of DENV fusion and virion maturation and an immune modulator of host response) [85], prednisolone (a corticosteroid that suppresses inflammation) [86], balapiravir (a cytidine analog that inhibits viral RNA synthesis) [87], celgosivir (an inhibitor of host α-glucosidase that modifies viral proteins essential for infection cycle) [88], and lovastatin (a cholesterol synthesis inhibitor) [89]. Unfortunately, none of the repurposed compounds showed any clinical benefits in dengue patients. It remains to be determined if the lack of efficacy is due to low potency of the compounds or because the treatment was too late in the course of disease. One potential way to differentiate between these 2 possibilities is to test the clinical candidates in a dengue human challenge model [90], which allows the start of drug treatment in a controlled manner. The human challenge model has been successfully used to evaluate dengue vaccine development [90]. Many of the challenges faced with DENV drug development will be applicable to ZIKV.

THERAPEUTIC ANTIBODIES

Therapeutic antibodies represent an attractive approach for ZIKV therapy. One potent human monoclonal antibody against viral envelope protein ZIKV-117 was reported to inhibit various strains from African and Asian-American lineages in cell culture. More important, this antibody exhibited efficacy in pregnant and nonpregnant mice. Treatment of ZIKV-117 antibody dramatically reduced tissue pathology, placental and fetus infection, and deaths in mice [91]. Another human monoclonal antibody, ZKA64, which binds to viral envelope domain III, was reported to protect A129 mice from lethal infection when given 1 day before or 1 day after infection [92].

A few issues remain to be addressed for further development of these promising antibodies. The first issue that needs to be addressed is the potential of antibody-dependent enhancement (ADE). Antibody-dependent enhancement has been reported for DENV, ZIKV, and other flaviviruses [93, 94]. Although transfusion of heterologous flavivirus antibodies to STAT knockout mice enhanced subsequent ZIKV infection [95], 2 subsequent studies did not support ADE in a human cohort and in nonhuman primates that had been previously exposed to heterologous flavivirus infection [96, 97]. Nevertheless, for mitigating potential ADE of therapeutic antibodies, mutations of L234A and L235A (known as LALA mutation) could be engineered into immunoglobulin G to prevent its binding to FcγR, which mediates the enhanced virion uptake into cells [98]. It should be noted that the LALA mutation may affect the transport efficiency of immunoglobulin G across the maternal–fetal placental barrier, therefore affecting its efficacy in pregnancy treatment [91]. On the other hand, recombinant antibodies could be engineered to increase their half-lives from approximately

20 days to 100 days, leading to antibodies that can maintain at an efficacious level for 6 months after a single injection [99]. Such long half-life antibodies would be ideal for prophylaxis prevention in pregnant women.

The second issue that needs to be addressed is resistance. Flaviviruses have been shown to rapidly develop escape mutants upon antibody treatment [100, 101]. Therefore, at least 2 antibodies will be needed in combination to minimize resistance emergence.

The third issue that needs to be addressed is the cost of goods. Even though the technology for antibody production has been significantly improved in the past decade, the cost of goods could still be prohibitive for their wide use, particularly in low-income countries. Their use may be further limited by the requirement of intravenous administration and cold-chain delivery from manufactures to patients.

CLINICAL DEVELOPMENT PATHS

Zika virus therapy could be administered at pre-exposure or postexposure time. Pre-exposure prophylaxis could be used for individuals living in households with known active infection/transmission and travelers to high-risk, epidemic areas. Similar to ring vaccination, prophylactic ring treatment could hinder the spread of virus by administration of it only to those who are most likely to be infected. Postexposure treatment could be administered to infected individuals, particularly pregnant women and newborns with active ZIKV infection. Therefore, an ideal antiviral drug will have to meet the unique pharmacologic criteria as a preventive or treatment option, as well as the safety criteria for pregnancy category A/B drugs.

There has been some success wtih using antiviral drugs and therapeutic antibodies to prevent vertical viral transmission during pregnancy. In the case of HIV, because the maternal viremia level correlates with the viral transmission rate to newborn babies, treatment with antiviral drugs during pregnancy reduces maternal viremia, leading to decreased mother-to-child transmission [102]. For herpes simplex virus (HSV), transmission of the virus from mother to fetus typically occurs by direct contact with HSV in the genital tract during birth. Antenatal antiviral prophylaxis reduces viral shedding and recurrences at delivery and reduces the mother-to-child transmission [103]. In addition, treatment of cytomegalovirus (CMV)–infected newborn infants with antiviral drugs significantly prevented hearing deterioration [104]. Besides small molecule drugs, therapeutic antibodies have also been tested to prevent and treat viral congenital infections in pregnant women with CMV, measles virus, and varicella zoster virus [105–108], among which hyperimmunoglobulin treatment significantly reduced congenital abnormalities in neonates [109].

Based on the above results in pregnant patients with HIV, HSV, or CMV, a 2-stage path could be conceived for ZIKV therapeutics development. The first stage is to demonstrate the safety and efficacy in nonpregnant patients. Viremia reduction in treated patients would be the key endpoint for initial efficacy elevation. To achieve this goal, recruitment of patients with early ZIKV infection is essential to evaluate a compound's antiviral efficacy; patients with late infection would not be useful for a compound efficacy test because their viremia would have already declined. Because ZIKV persists in the male reproductive tract for up to 6 months [31–33], infected males could be included for efficacy testing; the effect of a compound on male fertility should also be evaluated in parallel. Once clear safety and efficacy have been demonstrated in the first stage, the compound could advance to the second stage for development, with the goal of evaluating the safety and efficacy in pregnant women. Both pre-exposure prophylaxis and postexposure treatment could be pursued in pregnant patients.

In summary, although substantial progress has been made toward ZIKV drug discovery, the current pipeline remains at an early discovery stage. Once a candidate has reached phase I clinical trial, a proof-of-concept study could be achieved within 1–2 years if early infected, nonpregnant patients could be recruited to demonstrate antiviral efficacy. Once proof of concept has been achieved in nonpregnant patients, further development could be pursued to demonstrate efficacy in pregnant patients. It will take at least several years before ZIKV therapeutics become available for clinical use.

Notes

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