ZIKA DRUG DISCOVERY - A REVIEW

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Received: September 13, 2018 Accepted: October 29, 2018

ABSTRACT: Zika virus (ZIKV) has only recently been exposed as a significant public health threat, and much of our limited knowledge of its pathogenesis and triggered immune responses were discovered in only the last few years. There are currently no ZIKV-specific therapeutics or vaccines available. This review seeks to bring the reader up-to-date with the latest developments in finding a way to combat this emerging infectious disease. The developing fetus is most at risk of ZIKV complications, therefore pregnant women are an especially important target for treatments and vaccines; however, this presents challenges for ensuring both efficacy and safety in this immunocompromised population. Gaps in our ZIKV knowledge base, animal model limitations, and concern for adverse immune responses and teratogenic effects, all pose challenges to drug developers, and could prolong the wait before a ZIKV vaccine or treatment becomes available.

Key Words: Zika virus, disease, discovery, treatment.

Introduction

Zika virus (ZIKV) is an arbovirus that chimes to genus Flavivirus, which is aforethought to be the current emanant virus after the Ebola epidemic. (ZIKV) is a mosquito-borne virus transmitted by Aedes sp. mosquitoes across tropical and subtropical realms around the world. Despite the novel outbreak of Zika virus (ZIKV), there are still no affirmed treatments, and early-stage goulashes are probably many years away from approval. A discursive A–Z review of the recent overture in ZIKV drug discovery efforts is presented, highlighting drug repositioning and computationally guided compounds, including espied viral and host cell inhibitors (Al-Qahtani et al., 2016). It was first stranded from rhesus monkeys in Uganda, Africa, in 1947 (Weaver et al., 2016). In the past, ZIKV has only been couple with mild disease; however, after tame activity for six decades, it recently spurt as a facund threat to human health, with distinct fetal abnormalities, microcephaly, serious neurological complications, and autoimmune disorders such as Guillain–Barré syndrome (GBS) (Kruger, 2016). The rampant rate of spread ZIKV has drawn booming attention from worldwide scientific community for promoting candidate vaccines by investigating murine models and viral pathogenesis (Lazear et al., 2016). Computational accessions have been used for ZIKV drug discovery with fortuitous results. Besides the spurring of the discovery process, these techniques deflate experimental costs, ebb the use of animals in experiments and are environment-friendly (Ravinfranath et al., 2015). Advanced computational drug screening (the so called Virtual Screening techniques, or VS) such as docking techniques will play an important role in the espial of novel bioactive compounds in the context of ZIKV. The applicable crystal structures from parts of the virus and new homology models might be already used as an early stage of a computational protocol. More advanced docking techniques, including Blind
Docking simulations could be subsequently applied to transaction large databases of compound libraries (Fernandez et al., 2012). To date, little is understood about the tendency of ZIKV infection, and new modes of transmission, including sexual intercourse, as well as the appearance of severe aggravations, including birth defects and neurologic diseases, have only recently been limned. Newly established animal and cell culture models of ZIKV infection have bestowed some insight into the pathogenicity of ZIKV, but these systems are still in their naivety and have yet to reproducibly reiterate the more severe complications discerned in human infections. The paucity of dossier regarding the myriad aspects of Zika fever pathophysiology and the long-term consequences of pregnancy associated ZIKV infections have prompted a multinational stab to develop targeted therapeutics, effective vector control programs, and vaccines to sentinel vulnerable populations. Because of the virtually routine release of new publications pertaining to information about ZIKV and Zika fever, it is impossible to review the entire body of current knowledge (World Health Organization, 2014); however, we have attempted to summarize salient points regarding the epidemiology, basic biology, pathophysiology, diagnosis, treatment, and prevention of ZIKV infection

II. Virology

ZIKV and its imminent relative Spondweni virus comprise the Spondweni virus group within the genus Flavivirus, family Flaviviridae. Presently, there are 2 recognized lineages of ZIKV, African and Asian; the currently circulating strains in the Western Hemisphere loom to be derivatives of the latter. ZIKV particles, like other flaviviruses, are enveloped and spherical and slug approximately 50 nm in diameter (Fig. 2). Impacted in the lipid bilayer envelope of mature particles are 180 copies each of the membrane glycoprotein (M) and the envelope glycoprotein (E) that are sort in an icosahedron-like symmetry. With the exception of roughly 10 amino acid residues surrounding the Asn154 glycosylation site of each envelope glycoprotein.

Figure 2: Cartoon of ZIKV showing the enveloped and roughly spherical nature of the virus. Left, in cross-section, the single-stranded, positive-sense RNA genome, the capsid, and the envelope and its associated protein components are shown. Right, the proteins located on the surface (E and M) of a mature, intact particle are arranged in an icosahedron-like symmetry (http://viralzone.expasy.org).

The ZIKV infectious cycle (Fig. 3) drive with attachment of virions to the cytoplasmic membrane of susceptible cells, a trial mediated by interactions between the viral E protein and one or more host cell receptors, as well as dendritic cell specific intercellular adhesion molecule-3-grabbing non-integrin and members of the transmembrane immunoglobulin and mucin domain and Tyro-3, Axl, and Mer families of host cell receptors (Sirohi et al., 2016).

Figure 3: Flavivirus infectious cycle, a model for ZIKV replication. A virion attaches to the surface of a susceptible and permissive host cell via interactions between the viral E protein and one or more cell-surface receptors (A). The virion is next internalized via clathrin-mediated endocytosis and upon
acidification of the endosome, the viral and endosomal membranes fuse, releasing the viral genome into the host cell’s cytoplasm (B). Next, viral protein translation, RNA replication, and nascent particle assembly occur (C, D, and E), ultimately leading to the maturation and release of progeny particles from the cell (F and G) (Perera et al., 2008).

III. Pathophysiology

The tendency of ZIKV pathogenesis, the host’s immune response to ZIKV infection, and the mechanisms veiled sequelae are subjects of investigation by a very large, multidisciplinary group of researchers from around the world. Our accepted perspective of the Pathophysiology of ZIKV infection stems from a combination of in silico, in vitro, and in vivo studies, including the development of cell culture and animal models, post-mortem examination of human foetal and infant remains infected with ZIKV, as well as clinical case reports that cue never-before-seen complications yoke with ZIKV infection. Infection of primary human dermal fibroblast, primary human epidermal keratinocyte, and human dendritic cell cultures evince that all 3 cell types are susceptible and forbearing to ZIKV infection (Sirohi et al., 2016). It stands to reason that each of these cell types could latch on in the boosting of ZIKV at the site of inoculation and subsequent dissemination of the virus. This trance also decodes that infection of skin fibroblast cells sequel in activation of innate cellular antiviral responses. By way of experimental infection of human skin biopsy specimens with ZIKV, the histopathologic features of ZIKV in skin were also appraised (Perera-Lecoin et al., 2013). Studies escorted later, in 2016, demonstrated that ZIKV infects and nuke human neuronal stem cells grown as neurospheres and brain organoids, cognizance that helped solidify the link between foetal ZIKV infection and the development of microcephaly (Garcez et al., 2016). Numerous nonhuman primate and nonprimate animal species have been guesstimated to assess their propriety as models for ZIKV infection. It was also examined that the virologic and immunologic dynamics of ZIKV infection in rhesus macaques that were provisionally infected with Asian-lineage ZIKV in doses similar to those known to be dispatched by the bite of infected mosquito vectors (Dudley et al., 2016). Contrastingly, the kinetics of ZIKV ferreting out was similar to those portrayed in human patients, with RNA being detectable in blood, urine, and other body fluids. However, animal studies, including those using mice, have thrively recapitulated some pathologic features of ZIKV infection, together with the destruction of neuronal tissue (Li et al., 2016). Post-mortem analysis of ZIKV-infected human foetal and infant remains has sustained pivotal in the intimation of ZIKV in neuroteratogenesis. Histopathological inquest of infant and placental remains from cases of lethal congenital infection has so far validated that ZIKV antigens, intact viral particles, and nucleic acids are noticeable in the central nervous system tissues of microcephalic foetuses and placentas. In addition, the recognition of some infected cell types and affected anatomic regions (neurons and glial cells of infected infants, chorionic villi of placentas) was achieved by immune-histochemical staining for ZIKV antigens (Martins et al., 2016).

IV. Computational techniques applied to Zika drug discovery

The structural and biological dope about ZIKV is veritably vital for fortunately guiding drug discovery projects. However, the infrastructure stipulations for these experimental assays are beyond the reach of most researchers due to its high cost. To make experimental assays less plush, researchers can use computer-assisted drug discovery (CADD) as a complementary approach to poll compounds for synthesis and/or biological evaluation. As no compound need to be tested before computational simulations, CADD imitate a time, labour, and cost-effective strategy to obtain antiviral compounds in the early stages of ZIKV drug discovery projects (Ekins et al., 2016).

**Ligand-based drug design (LBDD)**

Usually, when the biological target is not known or its 3D structure is not available, CADD can use the chemical structures of known active compounds (i.e., obtained from biochemical or phenotypic assays) as starting points. This approach is known as Ligand-based drug design or LBDD (Glaab, 2016). There are four main LBDD methods:

(i) Similarity search; (ii) 3D shape matching; (iii) Ligand based pharmacophore and (iv) Quantitative-structure activity relationships (QSAR).

**Similarity search**

Ligand similarity search is based on the principle that structurally similar compounds exhibit similar biological activities. Figure 4 shows the schematic illustration of calculation of Ligand-based comparability using Tanimoto coefficient and bit strings (Cereto-Massagué et al., 2015). It speculates that two structures with Tanimoto coefficient higher than 0.85 can be aforesight structurally (Neves et al., 2016).
Ligand-based pharmacophores and shape-based models
Ligand-based pharmacophores and shape-based models aim to analyze potentially active compounds based on their overlapping to the 3D arrangement of key interacting chemical features (e.g., aromatic rings, hydrogen bond donors or acceptors, partial charges, etc.) or shape and volume of known active ligands not shared by inactive compounds (Figure 4A) (Caporuscio et al., 2011). In the same way, 3D shape-based models peg probably active compounds based on their overlapping to the 3D surface shape of active compounds (Figure 4B) (Hawkins et al., 2007). The spawning of both models basically involves four steps: (i) alignment of a training set of molecules (composed by actives and inactives) into a known bioactive query; (ii) scoring of compounds and culling of best pharmacophoric/shape hypothesis; (iii) statistical acceptance by using appropriate metrics to impel the ability of the hypothesis to discriminate between known active and inactive compounds (Braga et al., 2013); (iv) screening of untested compounds using the statistically ratified models.

Quantitative Structure-Activity Relationships (QSAR)
One of the most in vogue approaches for computer aided drug design is the quantitative structure-activity relationships (QSAR). QSAR is a method to determine the correlation between the chemical structures of a set of compounds and a intrinsic biological property (Gomes et al., 2017). QSAR modelling could be conferred as three-part process (Figure 4C). Initially, chemical structures are reformulated into molecular descriptors (independent variables), which are the result of logic and mathematical procedure that transforms chemical lope into a useful number (Todeshini et al., 2008). Then, machine learning methods (Breiman, 2001), Deep Learning (LeCun et al., 2015), Support Vector Machine (Vapnik, 2000) are used to entrench quantitative relationships between descriptors and biological property (dependent variable). This step involves empirically discovering a function that will establish weights to molecular descriptors reconcile the equation \( B_p = k' (D_1, D_2, \ldots D_n) \), where \( B_p \) is biological property of molecules, \( D_1, D_2, \ldots D_n \) are molecular descriptors and \( k' \) is some empirically established weight ascribed by the selected algorithm (Cherkasov et al., 2014). Once validated, the generated model can be used in VS campaigns to prioritize untested compounds for synthesis and/or biological assessment (Tropsha, 2010).

![Figure 4: Schematic representation of LBDD methods.](image)

Structure-based drug design (SBDD)
Structure-based drug design (SBDD) is a very useful access when the biological target is known, and its 3D structure is available. Based on the target 3D structural lore, SBDD methods can assist the druthers of ligands with good complementarity and affinity to the protein binding site (Lionta et al., 2014). In general, SBDD methods can be divided in three main categories: (i) protein-Ligand docking; (ii) structure-based pharmacophores; and (iii) molecular dynamics. These approaches are discussed below.
Protein-Ligand docking

Molecular docking is one of the most universally used SBDD methods, and largely applied in structure-based VS (SBVS) campaigns (Figure 5B). It reposes the computational fitting of ligands into the protein binding site, aiming to presage the Ligand-protein complex, and reckoning the Ligand binding affinity (Ferreira et al., 2015). In general, the docking process is accomplished in two main steps: (i) the exploration of the conformational space, by generating various poses (orientations) of the Ligand, which is executed by the search algorithm; and (ii) the use of scoring functions to percolate the ranking of the most promising Ligand poses, and estimate the binding affinity (Kalyaanmoorthy et al., 2011).

Structure-based pharmacophores

Structure-based pharmacophore modelling takes leverage of the information about the 3D structure of proteins, and uses this information to probe synergy points between the protein and the Ligand (Figure 5A) (Yang, 2010). There are two prospects of calculating a SBP from a 3D protein structure: (i) The protein structure is complexed with a Ligand (halo structure); and (ii) The protein has no complexed Ligand (Apo structure) (Pirhadi et al., 2013). Furthermore, it demands less computational cost in comparison to docking, and imitate a good alternative for researchers with limited computational resources (Braga et al., 2013).

Molecular dynamics simulations

Molecular dynamics (MD) simulations bestow detailed information about the decency of a system and its temporal evolution on a molecular scale. Through this technique; it's possible to incline the motion of proteins and molecules, at atomic level, and the distinct interactions among them (Salsbury, 2010).

In silico drug repurposing

The main intent of drug repurposing (or drug repositioning) is to establish new uses for already existing drugs (Ricci et al., 2016). This approach accelerates the drug discovery process, lessening time efforts and expenses, also circumvents preclinical development (Baker et al., 2018). Through this approach the following compounds have already been spotted as anti-ZIKV agents: nidosamide, PHA-690509, emricasan, seliciclib, bortezomib, mycophenolic acid, auranofin, ivermectin and daptomycin (March-vila et al., 2017). Today, only experimental HTS screening were enforced for ZIKV drug repurposing, but in silico methods are also a promising approach for ZIKV drug repurposing and have been prosperously applied in other examples (Barrows et al., 2016). This represents an opportunity that has been under-utilized for ZIKV.

Figure 5: Schematic representation of SBDD methods.
Here is a high content screening methodology for the discovery of inhibitors of ZIKV infection applied in a drug repurposing context. This assay was used to screen a library of FDA approved drugs, resulting in the assimilation of five compounds with selective activity against ZIKV in human cells.

V. Methods

ZIKV compound screening assay

The NIH Clinical Collection compound library (Evotec) was screened against ZIKV at 20 μM in 1% DMSO. MOCK-infected Huh7 and IFN 2A (1.55 nM) were used as positive controls, and the 1% DMSO (vehicle)-treated cells were used as negative control. In each run, a 10-point dose-response curve of the reference compound IFN 2A, starting at 1.55 nM and impoverished in a factor of 2, was also used for assay quality control. The compounds were diluted 16.6× in DPBS 1× in the μClear Black 384-well plates (Greiner Bio-One) for a final volume of 10 μL of compound at 6% DMSO. After that, 50 μL of a mixture of Huh7 cells at 6 × 104 cells/mL and ZIKV at a MOI of 0.5, were added in each well of the plate resulting in a final concentration of 1% DMSO and a final volume of 60 μL/well. After 72 h of incubation at 37°C and 5% CO2, the cells were submitted to indirect immunofluorescence (IF) protocol as distinguished below. The primary screening was enforced in two independent experiments and the confirmation ratio was calculated by the number of common hits in both assays divided by the total number of hits of the first assay using Pearson test in Graphpad Prism software, version 6. Scatter-plot apportioning of the entire screening was generated using Spotfire 7.0 (TIBCO) (Bruno et al., 2016).

Detection of infected cells by indirect immunofluorescence

The Huh7 cells were fixed with 4% (w/v) (PFA) (Sigma-Aldrich) for 30 min at room temperature, treated with 0.25% (v/v) Triton X for 15 min and incubated with the primary monoclonal antibody D1-4G2-4-15 (HB-112) primed in DPBS encompassing 2.5% FBS at 37°C for 2 h. After two wash steps with DPBS, plates were incubated with AlexaFluor594 conjugated goat anti-mouse IgG (Thermo Scientific) and 5 μg/mL of DAPI (4, 6 diamidino-2- phenylindole) (Sigma-Aldrich) in DPBS at 37°C temperature for 1 h, and then washed again twice with DPBS. After the final washing, digital images were acquired using a high content imaging system, the Operetta (Perkin Elmer). The digital images were taken from four different fields of each well at 20× magnification (Bruno et al., 2016).

Activity confirmation

To vouch the compound activity against Zika viruses, the selected hits from both primary screenings were tested in a 9 point DRC, with 2-fold serial dilutions starting at 50 μM, using the same assay and data analysis described for the primary screening. The EC50 value was used to estimate compound activity. The CC50 value, defined as the compound concentration resulting in a 50% reduction in cell viability compared with the infected IFN 2A treated cells, was used to evaluate cell toxicity. The compounds that conferred the Selectivity Index (SI), which is calculated as SI = CC50/EC50, equal or higher than 1 and that reached at least 50% of maximum activity were considered as confirmed hits. Here we describe a high content screening methodology for the discovery of inhibitors of ZIKV infection applied in a drug repurposing context. This assay was used to veil a library of FDA-approved drugs, resulting in the identification of five compounds with selective activity against ZIKV in human cells (Bruno et al., 2016).

Figure 6: Interface of image processing and analysis developed for the Zika High content screening.
VI. Discovery of new drugs with anti-Zika activity

This novel methodology tag five promising compounds, among 725 FDA-approved stew from the NIH Clinical Collection compound library. All the five herein identified active compounds are presently marketed drugs for distinct treatments. The molecular structure and pharmacokinetics data of the compounds are summarized in Table 1 and Table 2.

Table 1: Molecular structure of the five most promising compounds identified in the anti-Zika virus high content screening of the NIH Clinical Collection library (Bruno et al., 2016).

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Structure</th>
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<tr>
<td>Lovastatin</td>
<td><img src="image" alt="Lovastatin" /></td>
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<tr>
<td>5-Fluorouracil</td>
<td><img src="image" alt="5-Fluorouracil" /></td>
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<tr>
<td>6-Azauridine</td>
<td><img src="image" alt="6-Azauridine" /></td>
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<tr>
<td>Palonosetron</td>
<td><img src="image" alt="Palonosetron" /></td>
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Table 2: Summarized chemical and physical properties of the most promising compounds identified with anti-ZIKV activity [40].

<table>
<thead>
<tr>
<th></th>
<th>Lovastatin</th>
<th>5-Fluorouracil</th>
<th>6-Azauridine</th>
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<th>Kitasamysin</th>
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<tr>
<td>EC50 (μM)</td>
<td>16.3 ± 7.7</td>
<td>2.3 ± 0.1</td>
<td>14.3 ± 8.6</td>
<td>20.7 ± 8.6</td>
<td>41.7 ± 10.1</td>
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<tr>
<td>CC50 (μM)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Max. Activity (% of Infection inhibition)</td>
<td>97.7</td>
<td>88.3</td>
<td>57</td>
<td>60.7</td>
<td>69.1</td>
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<tr>
<td>S.I. (CC50/EC50)</td>
<td>&gt;3.06</td>
<td>&gt;33.33</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
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<td>Water Solubility (mg/mL)</td>
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<td>Very high</td>
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**VII. Conclusion**

ZIKV virulence and its devastating consequences to humans require that we quickly discover new antiviral to stop this virus. This brief review highlights the computational technologies that are readily available to academia and industry, including both LBDD and SBDD. These have the potential to accelerate the process of drug discovery for ZIKV. We emphasize that, parallel to the Computational strategies, it is important to perform the experimental validation and that there is a considerable opportunity for computational drug repurposing. Further the study developed here describes a high content screening assay which successfully identified five active compounds against Zika virus isolated in an area of high number of reported cases of newborn neural complications. Further investigation is needed to understand the mechanism of action responsible for the inhibition of the Zika virus infection. However, the molecules identified in this study are important starting points, since they can be further optimized to increase the efficiency inhibiting ZIKV infection. Moreover, based on the structure comparison, more than 4000 molecules where identified in the PubChem databank as analogs and structural variants which could be also be tested, and still more specific and potent compounds can still be identified or even designed.
References


7. World Health Organization, Pacific syndromic surveillance system, 2014; World Health Organization Western Pacific Region; 2014.


