Targeting chikungunya virus replication : insights into chikungunya virus replication and the antiviral activity of suramin in vitro

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Summary

This thesis is focused on understanding the particularities of Chikungunya virus (CHIKV) replication, and the mechanism(s) by which it can be inhibited by suramin, a compound with a broad spectrum of activity.

Chikungunya virus (CHIKV) is a member of the *Togaviridae* family, and it can be transmitted to humans through the bite of infected mosquitoes. In the past, this virus, of African origin, has caused large epidemics in Asia, but the most recent one took place in 2014-2015 in a new territory, the Americas, where it caused over 1 million suspected and confirmed cases. The infection is manifested through acute joint and muscle pain (that can last for years), fever, and rash. To this day, there is no approved vaccine or treatment for CHIKV infection. A much broader introduction regarding CHIKV and its genome organization, protein function, replication, pathogenesis and preventive or therapeutic strategies can be found in **Chapter 1**, the general introduction of the thesis. Soon after the CHIKV epidemic was over, another virus, also mosquito-borne and originating from Africa, continued to cause serious health problems in Central and South America. This was the Zika virus (ZIKV), a member of the *Flaviviridae* family. Though ZIKV mostly causes asymptomatic infections or mild disease characterized by low fever, rash, conjunctivitis, and malaise, the epidemics in the Americas have also linked ZIKV infection to fetal malformations like microcephaly and to Guillain-Barré syndrome in adults. Besides this, previously unrecognized routes of mother-to-child and sexual transmission were uncovered. Similarly, as in the case of CHIKV, no preventive or therapeutic strategy (vaccines or drugs) for treatment of ZIKV infection are available on the market. The compound suramin had been marketed for the treatment of parasitic infections, but its anti-cancer and antiviral potential were also discovered in the last 40 years. Therefore, we sought to test if suramin could inhibit the replication of CHIKV and ZIKV.

Chapter 2 describes the development of an *in vitro* replication assay (IVRA) that relies on CHIKV replication/transcription complexes isolated from infected cells. This assay can be used to study CHIKV RNA synthesis, as well as to identify inhibitors of this process and perform mode-of-action studies on these compounds. While optimizing the assay, we identified a new RNA species that is produced during CHIKV infection, alongside the genomic and subgenomic RNA that are required for the production of specific proteins involved in the replication and virus production process, but also in interaction with/manipulation of the host cell. The new species was called RNAII, similarly to the one identified for the CHIKV-related Sindbis virus (SINV) in 1997 by Wielgosz and Huang. It is suspected to direct the replication/transcription complexes to later in infection predominantly produce the subgenomic RNA, which is required for the production of structural proteins that are used for progeny virus assembly.

Using the IVRA, in **Chapter 3** we have identified suramin as a potent inhibitor of CHIKV replication. However, cell-based assays revealed that suramin's main mode-of-action is dependent on the inhibition of an early step of the replicative cycle, namely virus entry into the host cell. In addition, several suramin-related compounds were analyzed, and though these compounds were not more effective, they provided insight into the structural elements (symmetry of the molecule and the presence of negative charges) that are important for both inhibitory activities of suramin observed *in vitro* and in cell culture.

The antiviral effect of suramin is very broad, and in **Chapter 4** we show that it also inhibits the replication of the re-emerging ZIKV, by interfering both with its entry and biogenesis of progeny virions.

Subsequently, we explored how suramin can inhibit CHIKV entry steps. **Chapter 5** describes the identification of the CHIKV E2 envelope glycoprotein as the target of suramin and mode-of-action studies that suggest that by interacting with E2 suramin blocks virus attachment to cells, and subsequent fusion of the particle with cellular membranes. CHIKV can become more resistant to suramin by acquiring mutations in the E2 protein. The amino acid substitutions that we found were N5R and H18Q, which allowed the virus to replicate much better in the presence of suramin, as compared to the wild-type virus. However, a known CHIKV mutant, with a G82R substitution in E2 that adapts the virus for infecting mammalian cells (by interacting with a molecule on their cell surface, heparan sulfate), was more sensitive to suramin.

Lastly, **Chapter 6** summarizes and discusses the key findings presented in this thesis. Their implications in the context of the broader literature are presented, followed by a general discussion and conclusion.