

LYCORINE: PROSPECTIVE NATURAL CURE FOR ZIKA VIRUS INFECTION

Umoke Toochukwu

Villanova Polytechnic

umoketoochukwu@gmail.com

Abstract

In 2007, an epidemic of dengue-like illness ravaged the isolated island of the yap, federated state of Micronesia, located in the western pacific. Afterward, the centre for disease control and prevention (CDC) ascertained that the outbreak was as a result of the infection of a positive sense single stranded RNA arbovirus designated as 'Zika virus'. This viral infection has an adverse complication on the children and pregnant women. In the latter, it can lead to preterm birth and miscarriage. Although this pathogenic arbovirus represent a serious health burden in tropical and sub-tropical areas worldwide no therapeutic agent to protect susceptible individual age the viral infection is in existence; therefore, inhibitors of Zika virus are therefore needed. A benzyl phenethylamine alkaloid denominated as 'lycorine' has shown to exhibit some medicinal qualities such as anti-cancer, anti-plasmodial, anti-inflammatory, inhibition of hepatitis C virus replication and so others. Furthermore, studies have indicated that lycorine is capable of restraining the rdpd activities of Zika virus. Due to its potency and ability against Zika virus infection in vivo and in vitro, lycorine might offer promising therapeutic possibilities for combating the pathogenic virus in the near future.

Introduction

Zika virus disease is a zoonotic ailment induces by the infection of a susceptible host by Zika virus. Zika virus is an arbovirus from the flaviviridae family; the mosquito-borne virus was first isolated from a rhesus monkey in the zika forest of Uganda in 1947 (**Chen H., et al 2020**). Identification in human was in 1952 in Uganda and the United Republic of Tanzania (**World Health Organisation 2018**); the virus made its way into Nigeria in 1967. Zika virus is a positive sense, single stranded RNA virus transmitted to humans by mosquitoes. The virion is approximately 44mm in diameter with surface projection that measures roughly 5-10nm. The viral nucleocapsid is 25-30nm in diameter surrounded by a host-membrane derived lipid bilayer. Zika virus genome encodes three structural proteins (C, prM/M and E) and seven non-structural (NS) protein (NS1, NS2A, NS2B, NS3, NS4A, NS4B, AND NS5) (**Sirohi D., et al 2017**).

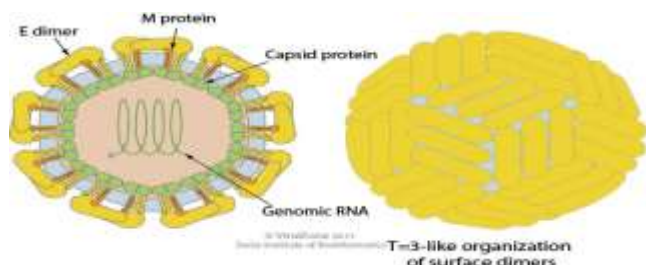


Figure1: diagram of zika virus

Zika virus is transmitted to people primarily through the bite of an infected *Aedes* species mosquito (*Ae. Aegypti* and *Ae. Albopictus*); these are same species of mosquitoes involve in the spread of dengue and chikungunya viruses. *Aedes spp.* Typically lay eggs in or near standing water, in things like buckets, bowls, animal dishes, flower pots and vases. They prefer to bite people and live indoors and outdoors near people; unlike anopheles mosquitoes, *aedes spp.* bite during the day. In addition to the arthropodean mode of transmission, ZiKV can also be transmitted from mother to fetus, through sexual intercourse, blood transfusion and also through laboratory and healthcare setting exposure (Centre for Disease Control, 2019).

Although, ZiKV infection leads to mild disease among some infected patients, adverse complications are evident in children and pregnant women. In the latter, it can spread to pregnancy defect such as fetal loss, stillbirth and preterm birth. In infants, zika virus infection can result to congenital zika syndrome; this syndrome includes microcephaly (a condition where baby's head is smaller than expected); brain damage and reduced brain tissue; eye damage; joint problem; limited motion; reduced body movement due to too much muscle tone after birth. In older children and adults, ZiKV infection is a trigger of Guillaine-Barre syndrome, neuropathy and myelitis. Amidst these adversities and complications, there are no therapeutic agent and vaccine to protect susceptible individual against the virus.

Nevertheless, nature has always been a perfect housing for the advent of promising therapeutic agents. Lycorine is a crystalloid alkaloid found in various Amaryllidaceae species such as the cultivated bush lily (*Clivia miniata*), surprise lilies (*lycoris*), and daffodils (*Narcissus pseudonarcissus*) (Lycorine, 2020). Lycorine is the major active component in *lycoris*, a represent of traditional Chinese medicine herb, and is one of the typical alkaloids with pyrrolphenanthridine nucleus core (Cao Z., et al 2013).

The benzyl phenethylamine alkaloid was first isolated from *Narcissus pseudonarcissus* in 1877 (Chen H., et al 2020); subsequently, it has been isolated and identified from alkaloids of other medicinal amarrylidaceous plants like *Lycoris radiata*, *Leucojun aestivum*, *Hymenocallis lithoralis*, *Ammodcharis coranica*, and *Brunsvigia radulosa* (Cao Z., et al 2013).

Structure of Lycorine

Structurally, lycorine is composed of molecular ring system derived from L-phenylalanine and L-tyrosine with a single nitrogen atom. Lycorine alkaloids have a typical pyrrolphenanthridine nucleus structure. The full chemical name of lycorine is 2,

4, 5, 7, 12b, 12c-hexahydro-1H-(1,3) dioxolo(4,5-j)-pyrrolo (3,2,1-de) phenanthridine-1-diol, and its molecular formula is C₁₆H₁₇NO₄ with the relative molecular mass of 287.31.

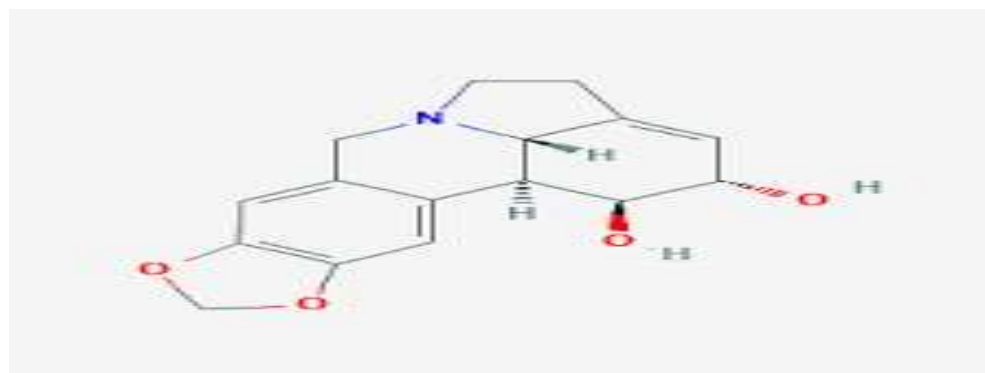


Figure 2: chemical structure of lycorine

The alkaloid is a colorless prism crystal with the melting point of about 260-262°C and it is very stable and can be preserved at room temperature for about 3 years. Lycorine is immiscible to the wastewater and insoluble in ether and alcohol. The hydrochloride form of lycorine is a needle-shaped crystal with the melting point of about 217°C.

Medicinal Properties of Lycorine

Based on accumulated evidence, lycorine have proven to exhibit multiple biological activities and pharmacological effects such as, anti-leukamia, anti-tumor, anti-angiogenesis, anti-virus, anti-bacteria, anti-inflammation, and anti-malaria (**Cao Z., et al 2013**). Lycorine has demonstrated its potentials to inhibit acetylcholinesterase and topoisomerase, suppression of ascorbic acid biosynthesis and control of circadian period. In addition, Lycorine inhibits growth of several *Saccharomyces cerevisiae* strains rho+, rho-, and mit-; whereas the strains devoid of mitochondrial DNA are resistant to lycorine at concentration of more than 200 µg/mL, suggesting mitochondrial genes are involved in the anti-bacterial effect of lycorine. Mechanism studies show that lycorine blocks the viralRNA replication and suppresses the viral protein synthesis. Lycorine strongly hinders RNA synthesis in flaviviruses and weakly inhibits viral protein translation. A gene allele substitution of viral 2K peptide in lycorine-resistant West Nile virus (WNV) confers the virus resistance to lycorine via an enhancement of viral RNA replication. Lycorine also inhibits the incorporation of [3H]leucine into poliovirus-infected HeLa cells and partially abolishes protein translation in the cells in a dose-dependent manner, implying that lycorine may impede protein synthesis in virus infected cells. Lycorine blocks the protein synthesis of the 3'-terminal region of the polyvirus translation unit, but not affect the protein synthesis of either 5'-terminal or middle region of the polyvirus translation unit. In addition, lycorine can completely protect mice from pathologic symptoms upon a low-dose EV71 virus challenge through blockade of virus polyprotein elongation during translation. Ribonucleoprotein (RNP) complex plays a pivotal role in

the viral generation and replication. Amaryllidaceae alkaloids do not affect the activity of the RNP complex, however, Amaryllidaceae alkaloid hemanthamine can suppress the migration of RNP from nuclear to cytoplasm, and lycorine delays the export of nucleoprotein protein from the nucleus. Collectively, lycorine is a potential candidate drug for anti-virus therapy.

Anti-Viral Activity of Lycorine on Zika Virus

According to the result of laboratory experiment conducted by Huini Chen and his team of scientist “qRT-PCR and plaque forming assay revealed that lycorine inhibits intracellular RNA synthesis and extracellular virion formation”. At a minute concentration of 0.5um, lycorine partially inhibited plague formation; complete inhibition was attained at concentration of 5um. Western blotting indicated that lycorine treatment caused a dose-dependent reduction in ZiKV protein, and envelope biosynthesis was significantly reduced in lycorine-treated cells in a concentration dependent manner.

Table: cell cytotoxicity and activiral of lycorine

Cell line	CC ₅₀ (μM)	Lycorine EC ₅₀ (μM)	SI
Vero	21	0.39	54
A549	4.29	0.22	19.5
Huh7	4.4	0.22	20

Source: the table was drafted from the result of Chen H., et al, experiment

The table above demonstrates the cytotoxic concentration 50% (CC50) values and 50% inhibition concentration (EC50) values in Vero (African green monkey kidney), Huh7 (hepatocellular carcinoma cells) and A549 (human alveolar basal epithelial adenocarcinoma) cells; it indicates that lycorine is a potent inhibitor of ZiKV infection in multiple cells.

Time of addition assay was used to clarify which step of ZiKV life cycle is affected by lycorine. The qRT-PCR results showed that 1um lycorine displayed the strongest ability to decrease the vRNA and viral progeny particle between -2 and 10hpi. Analysis of the infectious viral proteins in the cells revealed that lycorine reduced viral protein synthesis, which coincides with post-entry event of ZiKV replication cycle. Therefore, indicating that lycorine interfere with the translation and replication of vRNA. Futhermore, lycorine targets the synthesis of both positive and negative-strand RNA. Quantification test revealed that the levels of both positive and negative sense vRNA were decreased in lycorine treated cells compared to control cells. Lycorine inhibited – and + vRNA synthesis with efficacy of approximately 95% and 93% respectively. This observation suggests that lycorine inhibits the synthesis of positive and negative chains during viral replication.

CETSA was carried out to examine the direct interactions between lycorine and NS5. Tagg values of NS5 in Vero cell lysates were measured in the absence or presence of lycorine at temperatures ranging from 33 to 53.8 °C. The CETSA showed that lycorine treatment increased the thermal stability of NS5, markedly increasing the Tagg of the NS5 protein from 44.6 to 47.8 °C. These results confirmed that lycorine binds directly to NS5 and protects it from thermally induced aggregation.

Moreover, in vitro RNA assay confirmed that lycorine directly inhibits RdRp activity in a dose-dependent manner. In mice, ZIKV group displayed 100% mortality rate when treated with lycorine.

Conclusion

ZIKV infection is a serious threat to global health, and there are currently no approved drugs or licensed vaccines available for the prevention or treatment of ZIKV infection. Lycorine has divergent chemical structure and multiple biological functions and pharmacological effects, including anti-tumor, anti-tumor neovascularization, anti-virus, anti-bacterial, anti-inflammatory, anti-parasite, anti-plasmodia, and inhibition of ascorbic acid biosynthesis. The result of **Chen H., et al** experiment has proven lycorine to be an essential and effective arsenal against Zika virus infections.

Reference

- Cao Z., Yang P., and Zuou Q., Oct 2013; '*Multiple Biological Functions and Pharmacological Effects of Lycorine*'; Science China Chemistry, vol.56; no.10: 1382-1391
- Centre for Disease Control and Prevention 2019; U.S Department of Health and Human Services; accessed on Dec. 14th, 2020; <<http://www.CDC.gov/zikv/index.HTML>>
- Chen H., Lao Z., Xu J., et al (2020); '*Antiviral Activity of Lycorine Against Zika Virus in Vivo and in Vitro*'; Elsevier; Virology 546 (2020) 88-97, <www.Elsevier.com/locate/virology>
- Lycorine (2020, Dec. 5th). In Wikipedia, <<https://en.m.wikipedia.org/wiki/Lycorine>>
- Sirohi D., and Kuhn J.K., '*Zika Virus Structure, Maturation and Receptors*'; the Journal of Infectious Disease, Infection Society of America, HIV Medicine Association; 2016:216
- World Health Organisation 2018, accessed on June 13th, 2020, <<http://www.who.int/newsroom/fact-sheets/detail/Zika-virus>>