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Coordination of different ligands to copper(II) and cobalt(III) metal centers enhances Zika virus and dengue virus loads in both arthropod cells and human keratinocytes

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ABSTRACT

Trace elements such as copper and cobalt have been associated with virus-host interactions. However, studies to show the effect of conjugation of copper(II) or cobalt(III) metal centers to thiosemicarbazone ligand(s) derived from either food additives or mosquito repellent such as 2-acetylethiazole or citral, respectively, on Zika virus (ZIKV) or dengue virus (serotype 2; DENV2) infections have not been explored. In this study, we show that four compounds comprising of thiosemicarbazone ligand derived from 2-acetylethiazole viz., (E)-N-ethyl-2-[1-(thiazol-2-yl)ethylidene]hydrazinecarbothioamide (acetylethTSC) (compound 1), a copper(II) complex with acetylethTSC as a ligand (compound 2), a thiosemicarbazone ligand-derived from citral (compound 3) and a cobalt(III) complex with a citral-thiosemicarbazone ligand (compound 4) increased DENV2 and ZIKV replication in both mosquito C6/36 cells and human keratinocytes (HaCaT cells). Treatment of both cell lines with compounds 2 or 4 showed increased dengue viral titers at all three tested doses. Enhanced dengue viral plaque formation was also noted at the tested dose of 100 μM, suggesting higher production of infectious viral particles. Treatment with the compounds 2 or 4 enhanced ZIKV and DENV2 RNA levels in HeLa cell line and primary cultures of mouse bone marrow derived dendritic cells. Also, pre- or post treatments with conjugated compounds 2 or 4 showed higher loads of ZIKV or DENV2 envelope (E) protein in HaCaT cells. No changes in loads of E-protein were found in ZIKV-infected C6/36 cells, when compounds were treated after infection. In addition, we tested bis(1,10-phenanthroline)copper(II) chloride ($[Cu(phen)_2]Cl_2$, (compound 5) and tris(1,10-phenanthroline)cobalt(III) chloride ($[Co(phen)_3]Cl_3$, (compound 6) that also showed enhanced DENV2 loads. Also, we found that copper(II) chloride dehydrate ($CuCl_2{\cdot}2H_2O$) or cobalt(II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$) alone had no effects as “free” cations. Taken together, these findings suggest that use of Cu(II) or Co(III) conjugation to organic compounds, in insect repellents and/or food additives could enhance DENV2/ZIKV loads in human cells and perhaps induce pathogenesis in infected individuals or individuals pre-exposed to such conjugated complexes.

Importance: Mosquito-borne diseases are of great concern to the mankind. Use of chemicals/repellents against mosquito bites and transmission of microbes has been the topic of interest for many years. Here, we show that thiosemicarbazone ligand(s) derived from 2-acetylethiazole or citral or 1,10-phenanthroline upon conjugation with copper(II) or cobalt(III) metal centers enhances dengue virus (serotype 2; DENV2) and/or Zika virus (ZIKV) infections in mosquito, mouse and human cells. Enhanced ZIKV/DENV2 capsid mRNA or envelope protein loads were evident in mosquito cells and human keratinocytes, when treated with compounds before/after infections. Also, treatment with copper(II) or cobalt(III) conjugated compounds increased viral titers and number of plaque formations. These studies suggest that conjugation of compounds in repellents/essential oils/natural products/

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food additives with copper(II) or cobalt(III) metal centers may not be safe, especially in tropical and subtropical places, where several dengue infection cases and deaths are reported annually or in places with increased ZIKV caused microcephaly.

1. Introduction

Insect bites, food or crop damage and transmission of pathogens to humans, animals and plants has led to the extensive use of insecticides, pesticides and insect attractants or repellents [1,2]. Use of these substances has substantially been increased in military personnel and in agriculture or horticulture practices. Over a decade, several of these chemicals have been used extensively in all parts of the world. Some synthetic compounds or chemicals are of major concern to the environment and human health and they have been withdrawn due to the adverse effects and serious human health hazards [3–8]. One of the most recent study tested that several of the repellents including citronella candles had no effects and did not reduced mosquito attraction to humans [9]. The disadvantages of some insecticides have promoted the use of plant-based essential oils and their derived natural products, belonging to several herb species that poses repellent activities [7,10]. Some of the essential oils, herbal skin products and non-natural inorganic insecticides (the contact insecticides that are toxic to insects upon direct contact) have been shown to contain metals such as arsenates, copper, cobalt and fluorine compounds in small amounts and sulfur as a major component [11]. Also, both copper sulfate and cobalt chloride has been shown to improve the varieties in rice and vegetables that are mostly consumed in Australia and some parts of Asia [12]. In addition, complex mixtures of volatile organic compounds produced from plant-based essential oils or natural products such as citral have been extensively used as repellents to prevent transmission of human pathogens through biting arthropods such as mosquitoes [7,10,13].

Mosquitoes are the primary vectors responsible for the transmission of arthropod-borne flaviviruses that cause microcephaly (Zika Virus), dengue hemorrhagic fever, yellow fever, West Nile neuroinvasive disease (WNND) and Japanese encephalitis. With regard to the global impact from arthropod-borne diseases, dengue is the most important human pathogen (that exist as four serotypes; DENV-1, 2, 3 and 4) that should be addressed promptly. According to the 2009 WHO criteria, dengue cases have been classified according to the levels of severity. Dengue infections without warning signs or with warning signs (such as abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, liver enlargement, increasing hematocrit with decreased platelet numbers and lethargy) were considered as less severe. However, dengue infections with severe plasma leakage, bleeding or organ failures were considered as severe cases [14,15]. Approximately 20,000–25,000 deaths and 50–100 million dengue infections with 500,000 hospitalizations are reported, annually [16]. Also, there are no fully effective vaccines available for human use and prevention of dengue [17,18]. Nevertheless, recently, WHO immunization group (Strategic Advisory Group of Experts; SAGE) has recommended the use of partially effective dengue vaccine (a live attenuated tetravalent dengue vaccine developed by Sanofi Pasteur; CYD-TDV, named Dengvaxia) that has been licensed and used in 11 countries including Brazil, Mexico, Singapore, Thailand and Indonesia [19]. Related to dengue, Zika virus (ZIKV) is an emerging infectious agent transmitted and spread by the daytime active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*. ZIKV can be transmitted by blood transfusions, sexual and placental leakage from the mother to the fetus [20,21]. An important public health concern has been declared due to global epidemic of ZIKV infections. The association between ZIKV infection during pregnancy and intrauterine fetal infection, microcephaly, brain damage, congenital malformation syndrome and evidences from laboratory models of fetal infections are reported [20–22]. The best prevention practice is the use of mosquito nets, protective clothing such as long-sleeve shirts and full pants and insect repellents to avoid the mosquito bites. In high endemic areas, it becomes important to use repellents on a routine basis to prevent bites from infected mosquitoes.

Several reports suggest that trace elements such as copper and cobalt play an important role in the host immunomodulatory activities during viral infections [23,24]. Any changes in the levels of trace elements could impact disease severity [25]. Essential oils from plants and herbs can inactivate enveloped and non-enveloped viral replication and exhibit antiviral activities [26–29]. Due to the strong antiviral and antimicrobial activities, novel citral-based compounds (the well-known natural insect repellent) are developed continuously [26–29]. Studies that show the effect of conjugation of citral-based compounds with different trace elements on ZIKV and dengue virus replication has not been explored. The purpose of the current study is to analyze the effects of copper(II) and cobalt(III) metal conjugations with organic compounds (that are used in repellents or food additives) on DENV2/ZIKV infections. The four compounds analyzed in this study are selected based on the structural activity relationship (SAR) in order to determine the best efficacy in inhibition of the virus. It has been reported that when “free” thiosemicarbazone ligands are coordinated to a metal center, the efficacy in the activity is enhanced in comparison to the “free” ligands. In this study, we show that treatment of mosquito (C6/36 cells) and human keratinocytes (HaCaT cells), HeLa cells and primary cultures of mouse bone marrow-derived dendritic cells with thiosemicarbazones coordinated to Cu(II) or Co(III) metal centers enhances ZIKV and DENV2 infections. Our data suggests that use of coordinated version with metal centers in the repellent formulations may aid better replication of ZIKV and DENV2 in the human cells upon transmission by the biting mosquitoes.

2. Materials and methods

2.1. Synthesis of the ligands and the compounds

Analytical or reagent grade chemicals were used throughout this study. All the chemicals including solvents were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.) or other commercial vendors, and used as received. Microanalyses (C, H, N) were performed by Intertek Chemical and Pharmaceuticals, 291 Route 22 East, Salem Industrial Park, Building #5, Whitehouse, NJ 08888, U.S.A. The FT IR (Fourier Transform infrared) spectrum was acquired in the range of 4000–400 cm^{-1} using the Attenuated Total Reflectance (ATR) accessory (with a diamond crystal) on a Nicolet 6700 FT IR spectrometer. ^1H nuclear magnetic resonance (^1H NMR) spectra were acquired on a Bruker AVANCE III 400 MHz spectrometer with deuterated dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent. All ^1H NMR spectra were processed with the Spectrus Processor 2012 software, which is available from Advanced Chemistry Development (ACD, Inc., 8 King Street East, Suite 107, Toronto, Ontario M5C 1B5, Canada).

The “free” salts and complexes

The “free” $CuCl_2.2H_2O$ and $CoCl_2.6H_2O$ salts were obtained from Sigma-Aldrich Corporation.

Synthesis of (E)-N-ethyl-2-[1-(thiazol-2-yl)ethylidene]hydrazinecarbothioamide (acetylethTSC) 1 and [Cu(acetylethTSC)Cl]Cl·0.25C_2H_5OH 2 (Compounds 1 and 2, respectively)

(E)-*N*-ethyl-2-[1-(thiazol-2-yl)ethylidene]hydrazinecarbothioamide (acetylethTSC) 1 was synthesized and characterized as by Lewis et al.; [30] while the complex, [Cu(acetylethTSC)Cl]Cl·0.25C_2H_5OH 2 (where