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In Vitro Study: Effect of Cobalt(II) Chloride Against Dengue Virus Type 1 in Vero Cells

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Info Article	ABSTRACT
	Dengue virus (DENV) serotypes possess various types from DENV-1 to DENV-4 that are enveloped viruses belong to the genus Flavivirus of the Flaviviridae. Dengue vaccine or antiviral has not yet been clinically approved for humans, even though there have been significant efforts toward this end. Antiviral activity against DENV is needed to develop to be an alternative drug for the DENV virus. Cobalt(II) chloride has been used in the treatment and prevention of diseases of humans since ancient times. This study aimed to investigate the antiviral effects and Cytotoxicity of Cobalt(II) chloride. This compound was further investigated for its inhibitory effect on the replication of DENV-1 in Vero cells. The antiviral activity and cytotoxic was measured by WST-1 assay. The IC_{50} value of the Cobalt(II) chloride for DENV-1 was 0.38 µg/ml. The cytotoxicity of Cobalt(II) chloride to Vero cell suggests that the CC_{50} value was 2.91 µg/ml. The results of this study demonstrate the anti-dengue serotype 1 inhibitory activity of Cobalt(II) chloride was highly toxic. **Keywords:** Cobalt(II) chloride, DENV-1, Antiviral activity

INTRODUCTION

Dengue virus (DENV) is a positive-sense RNA virus replicating in the membranous compartments of cytoplasm. Viral infection induces the dengue virus with significant human viral pathogens transmitted by *Aedes aegypti*. Moreover, about 50 million infections occur each year, and over 2.5 billion people at risk (Gubler, 2002). There are four serotypes of the dengue virus. Four serotypes of dengue virus: DENV-1, DENV-2, DENV-3, and DENV-4) are transmitted by the vector mosquitoes such as *Aedes aegypti* and *Aedes albopictus* (Halstead, 2015). Vaccines have been developed for DENV infection (Woodland, 2015). 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 (Aguiar *et al.,* 2016). Nevertheless, control of the dengue virus through the use of vaccination has proved to be elusive

(Burke *et al.,* 2001). A new approach to controlling DENV infection is needed. Antiviral activity of DENV is required to develop an alternative drug for the dengue virus. The reason caused by the alternative drug is not an effective antiviral treatment for DENV, and the patient is not supportively-treated with any specific treatment measures (Zandi *et al.,* 2011).

Cobalt(II) chloride is an inorganic compound of cobalt and chlorine, with the formula $CoCl_2$. It is usually supplied as the hexahydrate $CoCl_2 \cdot 6H_2O$, which is one of the most commonly used cobalt compounds in the laboratory (Greenwood *et al.,* 1997). Cobalt is mainly found in the corrin ring of vitamin B12 (also known as cobalamin) and also supports metabolic and red blood processes (Chang *et al.,* 2010). Cobalt(II) chloride has been used in the treatment and prevention of diseases of humans since ancient times. It has been known for 45 years that small doses of cobalt produce polycythemia in many types of animals, including humans (Devlin *et al.,* 1967). In 1976, Duckham was investigated about the treatment of refractory anemia of chronic renal

Yovilianda Maulitiva Untoro[1]

failure with cobalt chloride. The research has shown that cobalt is a useful addition to the available therapy in the management of the refractory anemia of chronic renal failure (Duckham *et al.,* 1975). Enteric-coated cobalt chloride 25 mg twice daily is recommended for 12 weeks. The anemia will respond in the majority of patients during this time. It appears that within this period of treatment, the serum cobalt levels will reach a peak and level off. They suggest that either the maintenance dose of cobalt chloride is reduced to 25mg daily or that courses of three months be given intermittently.

In a previous study, Dutta *et al.* investigated the coordination of different ligands to copper (II), and cobalt(III) metal centers enhance the Zika virus and dengue virus loads in both arthropod cells and human keratinocytes (Dutta *et al.,* 2017). These findings suggest that the use of Cu(II) or Co(III) conjugation to organic compounds, in insect repellents and/or food additives could enhance DENV-2/ZIKV loads in human cells and perhaps induce pathogenesis in infected individuals or individuals pre-exposed to such conjugated complexes. However, there are just published data about anti-DENV type 2 activities of cobalt compounds. Therefore, the research aimed to determine the antiviral activity of Cobalt(II) chloride against dengue virus type 1 and the cytotoxicity of Cobalt(II) chloride to Vero cell.

MATERIALS AND METHODS

The chemical reagents used in this research were the Cobalt(II)-chloride (Sigma-Aldrich, Germany), dimethyl sulfoxide (Merck 99.98%, Germany), Minimum Essential Eagle Medium (Sigma-Aldrich, Germany), Dengue virus serotype 1 Surabaya Isolate (AB915377), Methanol (Merck 99.98%, Germany), Vero cell (African green monkey kidney), Viral ToxGlo Assay (Promega, USA), and Cell Proliferation Reagent WST-1 (Roche Applied Science).

Antiviral activity assay

Vero Cells with concentration 1×10^6 sel/10 mL were seeded into a 96 well plate and incubated plates containing cells at 37°C in a humidified CO_2 incubator for at least 4h (and up to 24h) to facilitate attachment and allow cells to recover from seeding stresses. One hundred microliters of Dengue virus with concentration 4×10^5 FFU/mL stock were combined with various concentrations of $CoCl_2$. The concentrations of $CoCl_2$ used in this study are 50μg/mL 25μg/mL, 12.5μg/mL, 6.25μg/mL, 3.13μg/mL, 1.56μg/mL (Zandi, 2011). After one hour of exposure to the dengue virus at room temperature, 100μL of the $CoCl_2$ treated virus was added to Vero cells in individual wells. Cells were infected for 24h. The unabsorbed virus was removed and replaced with MEM. Twenty-four hours post-infection 100μL of Viral ToxGlo. 100μL of ATP Detection Reagent was added to each well of a 96-well plate (25μL to each well of a 384-well plate) and wait at least 10min before measuring luminescence. Calculate IC_{50} values by plotting net RLU values (subtracting the average of blank wells) versus compound concentration. The IC_{50} value is the compound concentration that produced a 50% increase in ATP levels compared to virus and no-virus controls.

In vitro cytotoxicity assay

Cytotoxicity used WST-1 cell proliferation reagent by Roche Applied Science, Mannheim, Germany. The assay is very sensitive: it can detect 1,000 cells/well of a 96-well plate reader. Vero cells (1×10^5 cells/mL), 500μL of serial dilution compound, and a total of 10μL of Cell Proliferation Reagent was added to each well of a 96-well plate and incubated under 5% CO_2 at 37°C for 1h. The various concentration of $CoCl_2$ are 50μg/mL 25μg/mL, 12.5μg/mL, 6.25μg/mL, 3.13μg/mL, 1.56μg/mL (Zandi, 2011). The plate was read at 450nm (main filter) and 655 nm (reference filter) using an iMarkTM Microplate Absorbance Reader.

RESULT AND DISCUSSION
Antiviral activity

Antiviral activity is a homogeneous, add-mix-measure method intended that used in research studies to identify cytopathic effect (CPE) induced by a viral infection. Viral ToxGlo assay a more accurate quantitative measure of viral inhibition. Cells were either incubated with MEM, infected with dengue virus, or infected with $CoCl_2$ treated dengue virus. A significant inhibitory activity to that of the complex Cobalt (II) chloride was displayed against the tested pathogenic DENV-1 in Vero cells. In the inhibitory activity test, we studied the ability of the compound to produce a direct virus-inactivating effect. The basis of this test is the measurement of the ability of living cells based on mitochondrial activity from cell culture. This method is based on the ATP generated by active cells on a live-cell. Microplate reader calculated the live-cell after incubation for 24h.

The percentage inhibition of the development of dengue virus type-1 by the test sample of Co (II) chloride are presented on the figure (Figure 1).
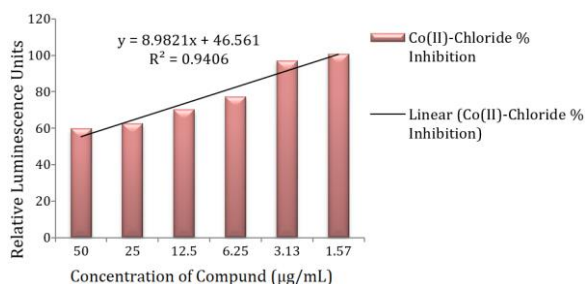

Figure 1. Inactivation of DENV-1 at variation concentrations of Cobalt(II) chloride

The mechanism analogy of inhibition DENV-2 from the previous study, it is also possible that an intact compound can diffuse across the cell membrane or virus capsid, or that Co(II) solute can enter cells through the transport and ion/voltage-gated channels. While Co(II) themselves can interact with oxidative organelles or redox-active protein to induce reactive oxygen species (ROS) in cells, Co(II) produced can also induce ROS by various chemical reactions, and ROS can break DNA strands and alter gene expression. Another possible mechanism is that Co(II) can chelate with biomolecules or dislodge the metal ions in some metalloproteins, leading to dysfunctional proteins and further cell inactivation (Broglie *et al.*, 2015).

The IC_{50} value was determined from the concentration-response curve (Figure 1); the IC_{50} value was 0.38μg/mL, R^2 was 0.9406. Based on the value of the IC_{50} Cobalt(II)-Chloride was a highly toxic compound. The antiviral activity of Cobalt has been described for distinct viruses. Delehanty *et al.* (2008) investigated the antiviral activity of Co(III)-hexammine to Sindbis virus replication. Co(III)-hexammine, significantly inhibited Sindbis virus replication in baby hamster kidney (BHK) cells in a dose and time-dependent manner. In plaque assays, the incubation of Co(III)-hexammine with Sindbis virus resulted in a dose-dependent decrease in virus replication when measured at both 24 and 48h post-infection Over the concentration range of 0–5mM Co(III)-hexammine, the IC_{50} for the inhibition of viral replication was determined to be 0.10±0.04mM at 48h. Analysis by flow cytometry confirmed that Co(III) hexammine mediated a concomitant dose-dependent increase in BHK cell viability and a decrease in the percentage of Sindbis virus-infected cells

(IC_{50}=0.13±0.04mM). These research findings demonstrate for the first time that Co(III) hexammine possesses potent antiviral activity (Delehanty *et al.,* 2008).

In the previous study, the majority of the inorganic ions tested enhanced the anti-herpes simplex virus type 1 (HSV-1) activity, EC_{50} (effective concentration) of $ZnCl_2$, $CuCl_2$, and $FeCl_3$ were 1.430μM, 121μM, and 35.8μM. All cations tested showed higher antiviral activity except for zinc. The mechanism of action was not determined during study, but evidence suggests that these chelates may target the extracellular attachment between the virion glycoprotein B and the heparin sulfate proteoglycans on the cells' surface (Langland *et al.*, 2018). The result of Co(III) complexes containing N, O donor ligands activity for HSV-1 has inhibited replication virus with as little as 5μg/mL required for active antiviral (Chang *et al.*, 2010). Many different metal ions are required to support the metabolism of cells. The suggestion has been made that all nucleotidyl transferases are metalloenzymes, and the involvement of zinc ions in both DNA and RNA polymerases is well documented. The attachment of a copper complex to an mRNA that is synthesized late in the infective cycle of viruses would affect the function of the mRNA, and this may account for the antiviral activity of these compounds (Hutchinson, 1985).
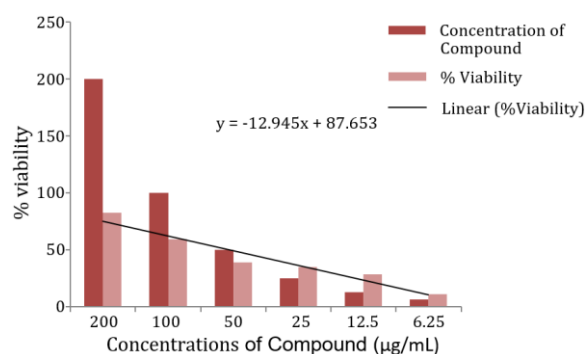

Figure 2 Cytotoxicity of Cobalt (II) chloride for Vero Cells at variation concentrations

Cytotoxicity of Cobalt(II) chloride to Vero Cell

Cobalt (II) chloride was screened for its cytotoxicity against Vero cells at different concentrations to determine the CC_{50} by WST-1 assay. The CC_{50} value was found to increase with an increasing concentration of the test compound (Figure 2). The CC_{50} of Cobalt (II) chloride for Vero cells was 2.91μg/mL. In this study, we have examined the relationship between the

Yovilianda Maulitiva Untoro[1]

concentration of Vero cells in the culture medium and the cytotoxic potency of Cobalt(II)–chloride.

In the previous study, Sucipto et al. investigated the antiviral activity of Copper(II) chloride dehydrate against dengue virus type-2 in Vero cell. It has been revealed that Co(II) is more toxic than Cu(II) with a CC_{50} value of 5.03μg/mL (Sucipto *et al.,* 2017). Copper(II) was non-toxic to human erythrocyte cells even at a concentration of 500μg/mL (Lv *et al.,* 2006). The effects of metal solutions containing Al(III), Cd(II), Hg(II), and Pb(II) to Vero cells were 6.25, 0.83, 3.7, 2.9, and 3.4μg/mL respectively (Traoré *et al.*, 1999). The $CoCl_2.6H_2O$ being the most effective antiproliferative agent, hence it was further tested against cancer cells and induced maximum cell death in IMR-32 followed by PC-3 and A549 with values of 7.12, 21.91, and 29.81μg/mL respectively (Mahey *et al.*, 2016). Thus, apoptosis was found to execute in cells by over generation of ROS (reactive oxygen species) and concomitant damage to the mitochondrial membrane.

For Vero cells incubated with polyoxometalate-stabilized gold nanoparticles for 24h was from 20-100μg/mL, approximately 93-95% was shown to be alive, 0.1-0.3% apoptotic. This data is commensurate with little or no toxicity when compared to the control sample (Gabas *et al.,* 2016). The synthesis of metal silver nanoparticles effect to Vero cell lines was found to be 18.15μg/mL. The cytotoxic effects had indicated the occurrence of active physicochemical interaction of silver atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA (Prasannaraj *et al.*, 2017).

CONCLUSION

Cobalt(II) chloride was shown to have antiviral activity against DENV-1, a member of the *Flavivirus* genus, *in vitro*. Antiviral activity results suggest that the IC_{50} value was 0.38 μg/ml. Cytotoxicity of Cobalt(II) chloride to Vero cell suggests that the CC_{50} value was 2.91 μg/ml. Based on the value of the IC_{50} and CC_{50} value, Cobalt(II) chloride was a highly toxic compound.

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