Inhibitory Activity of Cobalt(II)-Morin Complex Against The Replication of Dengue Virus Type 2

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Submission date: 19-Jul-2021 12:33PM (UTC+0800)
Submission ID: 1621412482
File name: INHIBITORY_ACTIVITY_OF_COBALT_II_MORIN_COMPLEX.pdf (393.53K)
Word count: 2740
Character count: 14456

Indonesian Journal of
Tropical and Infectious Disease
Vol. 6 No. 6 September–December 2017

Research Report

INHIBITORY ACTIVITY OF COBALT(II)–MORIN COMPLEX AGAINST THE REPLICATION OF DENGUE VIRUS TYPE 2

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ABSTRACT

Dengue virus (DENV) is a significant pathogen emerging worldwide as a cause of infectious disease. Antidengue treatments are urgently required to control the emergence of dengue. DENV is a mosquito-borne disease responsible for acute systemic diseases and serious health conditions. DENVs were distributed in the tropical and sub-tropical areas and transmitted to humans by Aedes agypty and Aedes albopictus. Dengue vaccine or antiviral has not yet been clinically approved for humans, even though there have been great efforts toward this end. Antiviral activity against DENV is an important alternative for the characterization and development of drugs. Metal–organic compounds were reported to exhibit fungicidal, bactericidal, and antiviral activities its inhibitory activity was not significant, at high concentration it was more toxic to replicating cells than to stationary cell monolayers of Vero cells. The aim of this study is to investigate the antiviral effects of Cobalt(II)–Morin complex. This compound was further investigated for its inhibitory effect on the replication of DENV-2 in Vero cells. The replication of DENV was measured by enzyme-linked immunosorbent assay and the value of selectivity index (SI). SI was determined as the ratio of the 50% cytotoxic concentration (CC_{50}) to the 50% inhibitory concentration (IC_{50}). The IC_{50} value of the Cobalt(II)–Morin complex for DENV-2 was 3.08 µg/ml, and the CC_{50} value of the complex for Vero cells was 3.36 µg/ml; thus, the SI value was 1.09. The results of this study demonstrate the antidengue serotype 2 inhibitory activity of Cobalt(II)–Morin complex and its high toxicity in Vero cells. Further studies are not required before Co(II)–Morin can be applied in the treatment of DENV-2 infections.

Keywords: *cobalt(II), morin, complex compound, inhibitory activity, DENV-2*

ABSTRAK

Virus Dengue (DENV) adalah patogen yang muncul secara global pada penyakit menular. Pengobatan anti-demam diperlukan untuk mengendalikan demam berdarah. Virus Dengue (DENV) adalah penyakit yang ditularkan melalui nyamuk atas penyakit sistemik akut dan kondisi kesehatan yang memilukan. DENVs didistribusikan di daerah tropis dan sub-tropis dan ditransmisikan ke manusia oleh agregat Aedes dan Aedes albopictus. Kini, vaksin dengue atau antivirus untuk manusia tidak disetujui secara klinis, meski telah ada upaya besar untuk mencapai tujuan ini. Aktivitas antiviral melawan DENV merupakan alternatif penting untuk karakterisasi dan pengembangan obat-obatan. Senyawa organik-logam dilaporkan menunjukkan aktivitas fungisida, bakterisida, dan antivirus, aktivitas penghambatannya tidak signifikan, dengan konsentrasi tinggi, lebih beracun untuk mereplikasi sel daripada monolay sel steroid sel Vero. Tujuan dalam proyek ini adalah investigasi senyawa antiviral kompleks Kobalt (II) -Morin diuji lebih lanjut untuk efek penghambatan pada replikasi DENV-2 pada sel Vero. Replikasi virus dengue dilakukan dengan metode enzyme-immunosorbent assay (ELISA) dan nilai indek selektifitas (SI), SI ditentukan sebagai rasio konsentrasi sitotoksik 50 (CC_{50}) terhadap konsentrasi hambat 50 (IC_{50}) untuk senyawa. Nilai IC_{50} Cobalt (II) -Morin untuk virus dengue tipe 2 adalah 3,08 µg / ml, dan nilai CC_{50} Cobalt (II) -Morin

untuk sel Vero adalah 3,36 µg / ml; demikian nilai SI untuk Cobalt (II)-Morin adalah 1.09. Hasil penelitian ini menunjukkan bahwa Cobalt (II) -Morin menunjukkan aktivitas penghambatan serotipe 2 anti-dengue dan memiliki sifat beracun pada sel Vero. Studi lebih lanjut tidak diperlukan sebelum Co (II)-Morin dapat diterapkan dalam pengobatan infeksi DENV-2.

Kata kunci: *Kobalt(II), Morin, Senyawa Kompleks, Aktivitas Penghambatan, DENV-2*

INTRODUCTION

Dengue virus (DENV) serotypes DENV-1–DENV-4 are enveloped viruses that belong to the genus *Flavivirus* of the Flaviviridae; it is widespread in the tropical and subtropical areas globally. The World Health Organization reported that the incidence of dengue increased 30-fold in the last five decades, and it is estimated that about 390 million people are infected with DENV worldwide.[1] Many efforts have been made to prevent and treat DENV infection, and clinical trials of a number of vaccines are currently underway.[2] Antiviral activity against DENV is an important alternative for the characterization and development of drugs. Complementary to vaccine, inhibitors of any natural step of the virus's replicative cycle have the potential for the treatment of DENV infection and indeed compounds such as inhibitors of RNA replication are already tested as such.[3] However, there is no drug commercially available yet with antiviral activity for DENV.[4]

Morin or 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one is a flavonoid that exhibits various biological activities, such as anti-*Bacillus cereus* and anti-*Salmonella enteritidis*,[5] antioxidant,[6] anti-inflammatory,[7] and antiviral for *equid herpesvirus* 1.[8] However, the antiviral activity for DENV has not been reported yet.

Metals have been used in the treatment and prevention of diseases of humans since ancient times. Already in 2500 BC in China elemental gold was in use as therapy for certain diseases, so-called chrysotherapy.[9] Gold and the more recently developed nano-gold have already had a large impact on medicine, especially in HIV therapy and cancer treatment.[10] Metal–organic compounds were reported to exhibit fungicidal,[11] bactericidal,[11–14] and antiviral[13,15] activities.

In a previous study, ribavirin was shown to exert its toxicity by inhibiting the intracellular energy metabolism and oxidative membrane damage, leading to accelerated extravascular hemolysis by the reticuloendothelial system. Although its inhibitory activity was not significant, at high concentration it was more toxic to replicating cells than to stationary cell monolayers of Vero cells.[16] Currently, there are no published data on the possible anti-DENV activities of cobalt compounds. In the present study, the inhibitory activity of Cobalt(II)–Morin complex against the replication of DENV-2 in cell culture was investigated.

MATERIAL AND METHOD

Chemicals and Media

The chemical reagents used in this research were the Cobalt(II)–Morin complex compound, dimethyl sulfoxide (Merck 99.98%, Germany), Minimum Essential Eagle Medium (Sigma-Aldrich, Germany), dengue virus serotype 2 Surabaya Isolate (KT012513), Vero cell (African green monkey kidney), CellTiter96® Non-Radioactive Proliferation reagent (Promega, USA), and DENV antibody (4G2) for enzyme-linked immunosorbent assay (ELISA).

Antiviral Activity Assay

Confluent monolayers of Vero cells were prepared on a 96-well plate (1×10^6 cells/10 ml) and counted using a hemocytometer, and the titer of DENV-2 (2×10^4 FFU/well) was expressed in Foci-Forming Units (FFU) after incubating at 37°C for 2 days. The 50% inhibitory concentration (IC_{50}) was calculated as follows: $IC_{50} = (NC - AC) \times 100/NC$, where NC is the mean of the number of negative controls and AC is the absorbance of the compound tested. The inhibition of DENV-2 replication by each compound was further investigated by using quantitative ELISA.

Cytotoxicity Assay

A cytotoxicity assay was performed using CellTiter96® Non-Radioactive Proliferation reagent. The CellTiter96® Assay is a modification of the MTT assay method described by Mosmann.[17] 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), 500 µl of serial dilution compound, and a total of 100 µl of Cell Proliferation Reagent was added to each well of a 96-well plate and incubated under 5% CO_2 at 37°C for 1–4 hours. The plate was read at 570 nm using an iMark™ Microplate Absorbance Reader.

RESULTS AND DISCUSSION

Antiviral Activity of Cobalt(II)–Morin

A significant inhibitory activity to that of the complex Cobalt(II)–Morin was displayed against the tested pathogenic DENV-2 virus in Vero cells. In the inhibitory activity test, we studied the ability of the compound to produce a direct virus-inactivating effect. The IC_{50} value was determined from the concentration–response curve (Figure 1); the IC_{50} value was 3.08 µg/ml, R^2 was 0.9404,

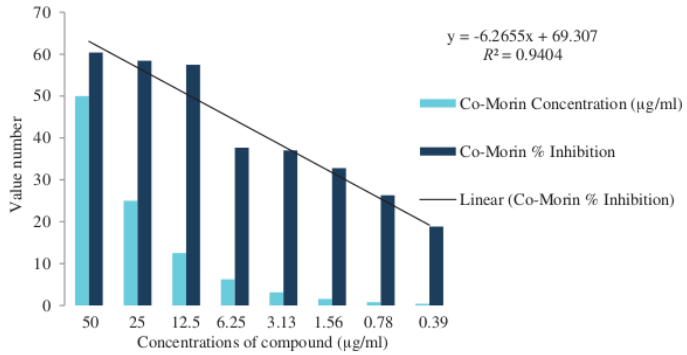

Figure 1. Inactivation of DENV-2, a member of the *Flavivirus* genus, at several concentrations of Cobalt(II)–Morin

and the value of the selectivity index (SI) was 1.09. The SI of the antiviral compound appeared to be moderately influenced by the strain of DENV tested.[18]

The emergence of arboviruses worldwide raises the necessity of developing a new strategy for the treatment of the diseases they cause. Several approaches have been demonstrated for the treatment of DENV-2 in which Cobalt(II)–Morin at concentrations of micrograms per milliliter promote inactivation. The antiviral activity of natural and synthetic Morin has been described for distinct viruses.[8,19] In members of the Flaviviridae family, the activity of Morin has been described for the replication system of Canine distemper virus). Here, the IC_{50} value of Morin is 40.52 ± 1.69 µg/ml for a 1-hour incubation. Investigation of Morin's structure showed that the compound is able to inhibit the adsorption and penetration stages.[19]

In HepG2 cells, the protection conferred by heme and Co(II)–protoporphyrin IX seems to be the result of decreased DENV-2 replication upon treatment. The IC_{50} value was reported to be 3.912 ± 1.4 µmol/l. Treatment of THP-1 cells with Co(II)–protoporphyrin IX, after infection

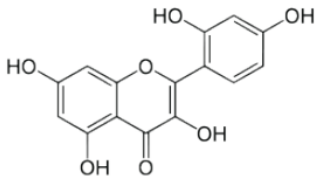

Figure 2. Structure of Morin

at a low multiplicity of infection showed similar results regarding cell viability and DENV replication after 72 hours post inoculation. This *in vitro* studies indicate a potential therapeutic use porphyrins in the treatment of flavivirus infection.[4]

Figure 2 shows the structure of Morin. Morin has hydroxyl groups at C-2′ and C-4′ (*meta* position). The activity of Morin might be related to the position of the hydroxyl groups at C-2′, which might prevent its biological effects on virus. However, the specific mechanism of Co(II)–Morin is still unclear.

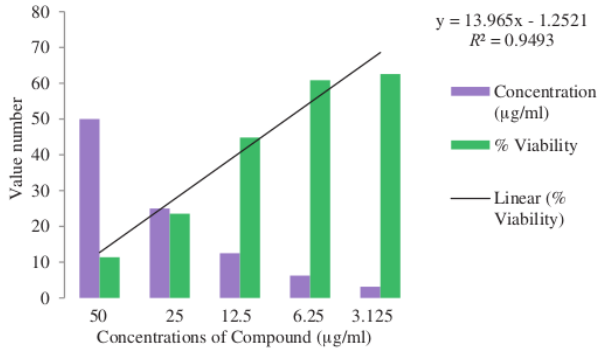

Figure 3. Cytotoxicity of Cobalt(II)–Morin for Vero Cells at several concentrations

Cytotoxicity of Co(II)–Morin to Vero Cells

The cytotoxic activity of Co(II)–Morin was investigated. This compound was tested against Vero cells at different concentrations to determine the CC_{50} value using CellTiter96® Non-Radioactive Proliferation reagent. The CC_{50} value was found to increase with an increasing concentration of the test compound, as shown in Figure 3. The CC_{50} of Cobalt(II)–Morin for Vero cells was 3.36 µg/ml, with an R^2 value of 0.9493. In this study, we have examined the relationship between the concentration of Vero cells in the culture medium and the cytotoxic potency of Cobalt(II)–Morin.

Cobalt(II) is stable in water by coordinating to ligands or chelators and is more stable than Co(III).[13] When compared with a previous study, it has been revealed that Co(II) is more toxic than Cu(II) with a CC_{50} value of 5.03 µg/ml.[18] Copper(II) was found to be nontoxic to human erythrocyte cells even at a concentration of 500 µg/ml.[20]

CONCLUSION

Further studies are not required before Co(II)–Morin can be applied in the treatment of DENV-2 infections. This study did not show the potential of the Co(II)–Morin complex as a candidate for antiviral agent against DENV-2 because it was shown to be toxic to Vero cells.

ACKNOWLEDGEMENT

This research was supported by the joint program of the Japan Initiative for Global Research Network on Infectious Disease (J-GRID); a Research Grant from Mandat Universitas Airlangga (HRMUA); the Institute of Tropical Disease (ITD); the Center of Excellence (COE) program by the Ministry of Research and Technology (RISTEK) Indonesia; and the Chemistry Department of Universitas Airlangga.

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