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Antiviral and anti-inflammatory activities of favipiravir and quinine sulfate against dengue virus serotype 1 in vitro

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ABSTRACT

Dengue virus (DENV) infection is still a global health problem. The severity of DENV infection is related to the high viral load and cytokine storm caused by excessive inflammation. There is no specific antiviral used for DENV. Meanwhile, the use of anti-inflammatory drugs for DENV is limited to patients with severe clinical symptoms. Interestingly, favipiravir (FVP) and quinine sulfate (QS) have been reported as repurposing drugs that can inhibit DENV replication. However, their anti-inflammatory activity in DENV infection has not been studied yet. Thus, the current study aimed to evaluate both antiviral and inflammatory activities of FVP and QS in Vero and PBMC cells. The FVP and QS antiviral activities were analyzed through half-maximal inhibitory concentration (IC_{50}) and half-maximal cytotoxicity concentration (CC_{50}) values against DENV serotype-1 on Vero cells. The anti-inflammatory activities of FVP and QS were measured by the relative expression of TNF-α, IL-6, IL-10 cytokines, and the transcription factor NF-κB from DENV-1 infected peripheral blood mononuclear cells (PBMC) in vitro. The results showed that the IC_{50}, CC_{50}, and selectivity index (SI) for FVP were 2.72 μg/mL, 156.78 μg/mL, and 58, respectively. Meanwhile, the IC_{50}, CC_{50}, and SI of QS were 14.97 μg/mL, 85.2 μg/mL, and 5.69. Also, FVP and QS reduced the expression of IL-6 and IL-10 but induced the expression of TNF-α, and the transcription factor NF-κB in PBMC with the presence of antibody-dependent enhancement (ADE). Further, FVP has better DENV-1 antiviral activities compared to QS. However, in comparison with QS, FVP showed lower anti-inflammatory activities. Further studies are needed to explore the antiviral and anti-inflammatory mechanism of FVP and QS in the DENV-infected models.

INTRODUCTION

Dengue hemorrhagic fever (DHF) remains a serious disease in tropical and subtropical regions of the world. According to the CDC, 400 million people worldwide are infected with dengue each year. Approximately 100 million people become ill, and 40,000 die from severe dengue [1]. It was reported that the confirmed cases of dengue in Indonesia were 143,266 and accounted for 1,237 deaths in 2022 [2].

DHF caused by dengue virus (DENV) infection belongs to the Flaviviridae family and has 4 different serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) [3-6]. All four serotypes exhibit considerable similarity, with approximately 65% of their genomes being identical [7]. DENV is transmitted through the bite of *Aedes* sp. mosquitoes (*Ae. Aegypti* and *Ae. Albopictus*) and causes no symptoms (asymptomatic) or symptoms (symptomatic). Symptomatic dengue cases are classified as undifferentiated febrile illness (UF), dengue fever (DF), DHF, and dengue shock syndrome (DSS). DF patients present some clinical symptoms such as headache, bone pain, and low-grade fever, so it

is rarely fatal. DHF patients differ from DF in that DHF patients have plasma leakage. In the case of DSS, the clinical picture is similar to DHF, but the plasma leakage is very severe and causes the patient to go into shock [8].

The pathogenesis of DENV infection involves both viral and host factors. Host factors in the form of immune responses such as autoimmunity, antibody-dependent enhancement (ADE), and T cells trigger the emergence of cytokine storms [9, 10]. Cytokine storm is a state of increased production of excess cytokines in a short period of time due to an imbalance between Th1 and Th2 cytokine responses [11-13]. Dengue-infected patients have elevated levels of pro-inflammatory (TNF-α, IL-6, IL-8, and IFN-γ) and anti-inflammatory (IL-10) cytokines [14]. Cytokine profiles can be analyzed using DENV-infected peripheral blood mononuclear cells (PBMC) [15]. PBMC consists of lymphocytes (T cells, B cells, and NK cells), monocytes, and dendritic cells with percentages of 70-90%, 10-20%, and 1-2%, respectively [16].

There is no specific treatment for DENV infection, only supportive care such as antipyretics, intravenous rehydration, and, in special circumstances, platelet transfusion are available [17]. Antiviral drugs specific for DENV with the ability to reduce inflammation have been developed [18, 19]. However, no antivirals have been approved for the treatment of DENV. Drug repurposing is a breakthrough therapeutic against infection. Therapeutically, the use of marketed repurposing drugs with antiviral activity in DENV infection is a strategy that can reduce the risk, time, and cost associated with drug development and is also highly effective [12, 20, 21].

Favipiravir (FVP) is a prodrug that is converted to its active form, favipiravir ribofuranosyl-triphosphate (FTP), by targeting the RNA-dependent RNA polymerase (RdRp) of viral RNA, resulting in the cessation of viral replication and mutagenesis [22]. FVP also reduces DENV titers without cell toxicity effects [23]. In addition, quinine sulfate (QS) is an antimalarial drug in the form of an alkaloid compound derived from cinchona bark, which inhibits RNA virus replication. Quinine increases the synthesis of RIG-I and IFN-α and blocks translation by activating protein kinase R and mRNA degradation by activating RNAse L, which inhibits RNA replication [24, 25]. In dengue treatment, quinine inhibited replication of all serotypes of DENV. However, the internalization process of DENV into host cells could not be hampered [26]. In repurposing drugs, an experimental approach is needed to screen and prove the effectiveness and safety of the tested drugs [27]. Therefore, a dose-dependency test and a cytotoxicity test were conducted in this study. Considering that the anti-inflammatory effects of FVP and QS in dengue fever have never been tested, it is necessary to conduct testing. Therefore, this study aimed to determine the antiviral and anti-inflammatory effects of repurposing drugs FVP and QS in DENV-1 in vitro.

MATERIALS AND METHODS

Drugs

The FVP was purchased from Toyama Chemical Co., LTD under the brand name Avigan. The QS drug was bought from PT Kimia Farma Tbk, Indonesia. 10 mg of FVP and QS was dissolved in 1 mL of 100% dimethyl sulfoxide (DMSO) as a stock solution with a final concentration of 10 mg/mL. The stock solution was then used to make various concentrations of 0.3125, 0.625, 1.25, 2.5, 5, 10, 20, 40, 80, 160 and 320 µg/mL in MEM + 2% FBS medium.

Cell culture

Vero E6 C1008 cells were cultured in Gibco™ minimum essential medium (MEM) (Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) and 0.5% antibiotic-antimycotic at 37°C in a 5% CO_2 incubator.

Isolation of virus

The virus used was Dengue serotype 1 (DENV-1) Strain IDS 11/2010, isolated from patients infected with DENV-1 in 2010 by Dengue researchers at the Department of Microbiology, Faculty of Medicine Universitas Indonesia, and propagated in Vero E6 cells. Virus titration was performed by Focus Forming Assay (FFA) in Vero E6 C1008 cells [28, 29]. Vero E6 C1008 cells were seeded in 96-well plates using 10% FBS MEM with a cell concentration of $2x10^4$ cells/well. Supernatants from DENV-1 cultures were diluted in various dilutions from 10^{-1} to 10^{-5} and infected to the cells. Then, incubated at 37°C in a 5% CO_2 incubator for 2 h with agitating every 30 minutes. After incubation, the wells were given 1.25% methylcellulose and incubated the plate at 37°C and 5% CO_2. After incubation for 2 days, infected cells were fixed by adding 3.7% formaldehyde. After washing with 1x non-sterile PBS 3 times every 5 minutes, added with Triton-X 0.5% (Sigma Aldrich, USA) and incubated for 20 minutes. Then, the primary antibody at a dilution of 1/1000 in 1% bovine serum albumin (BSA; Sigma-Aldrich, USA), 100 uL/well was added and incubated for 1 h. After washing, cells were then added with horseradish peroxidase (HRP) labeled secondary antibody (Goat anti-Human IgG (H+L); Invitrogen, Thermo Fisher Scientific, USA) at a dilution of 1/1000 in 1% BSA, 100 uL/well and incubated for 1 h. Diaminobenzidine 1x substrate (DAB; Thermo Fisher Scientific, USA) as substrate was added to the wells and incubated for ± 15 minutes. Foci as dark brown dots were observed under an inverted microscope [28].

This study was conducted at the Laboratory of Virology and Molecular Biology, Department of Microbiology and Infectious Disease and Immunology Research Center, Faculty of Medicine, Universitas Indonesia. Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, University of Indonesia (No. KET-1449/UN2.F1/ETIK/PPM.00.02/2023).

Determination of IC_{50}

The anti-DENV activity assay was performed by seeding Vero E6 C1008 cells at a concentration of 2 x 10^4 cells/well in a 96-well plate as in the previous study [28] to determine the half-maximal inhibitory concentration (IC_{50}). Viruses with Multiplicity of Infection (MOI) close to 0.5 FFU/cell were mixed with the FVP with concentrations of 0.3125, 0.625, 1.25, 2.5, 5 and 10 μg/mL. In the case of QS, concentrations of 5, 10, 20, 40, 80, and 160 μg/mL for the anti-DENV activity assay were used. In both cases, every concentration was replicated 3 times. 0.1% DMSO was included in the assay as a negative control. The drug-virus mixture was then inoculated into monolayer Vero E6 C1008 cells and incubated for 2 h at 37°C in a 5% CO_2 incubator, with plate agitation every 30 minutes. The supernatant of the plate was replaced with MEM containing 2% FBS and drug at the appropriate test concentration and then incubated for 48 h at 37°C in a 5% CO_2 incubator. After 48 h, the supernatant was harvested for further titration.

Determination of CC_{50}

The MTT assay was performed by seeding Vero E6 C1008 cells at a 96-well plate with a concentration of 2 x 10^4 cells/well to determine the half-maximal cytotoxic concentration (CC_{50}). Cells were then treated with FVP at concentrations of 5, 10, 20, 40, 80, and 160 µg/mL, while QS at concentrations of 0.625, 1.25, 2.5, 5, and 10 µg/mL. Both are processed in triplicate and incubated for 48 h at 37°C with 5% CO_2. 0.1% DMSO was used as a negative control. After adding MTT solution and 100% DMSO, absorbance readings were taken at a wavelength of 490nm [30]. The optical density (OD, absorbance) of each well in the indicated groups was used to calculate the percentage of cell viability as follows: percentage of cell viability = (*A* treatment – *A* blank)/(*A* control – *A* blank) × 100% (where, *A* = absorbance).

Anti-inflammatory assay

Anti-inflammatory activity was performed with PBMC cultured in 96-well plates at a concentration of 2x10^5 cells/well in triplicate [15]. After informed consent, 12-20 mL of the blood was drawn from human subjects with inclusion criteria of having no fever for the last 3 weeks, negative DENV infection, negative NS1 antigen, and anti-dengue IgM antibody to obtain PBMC. PBMC were cultured in Roswell Park Memorial Institute (RPMI) medium with 10% FBS, and 1% antibiotic-antimycotic. We used Concanavalin-A, a mitogenic stimulated agent, as a positive control of inflammation. In purpose to infect PBMC with DENV-1, DENV-2-infected patients with a dilution of 1/10,000 as ADE was used [31]. PBMC were incubated at 37°C with 5% CO_2 and were treated as indicated in Table 1.

After incubation for 24 h, PBMC in 96-well plates was extracted to obtain cellular RNA to determine pro- and anti-inflammatory cytokine expression. RNA was converted into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Cytokine analysis was performed by Real-Time PCR using PowerUp™ SYBR™ Green Master Mix for qPCR reagents (Applied Biosystems, USA). We used primers from previous studies (Table 2). Relative mRNA expression was analyzed against housekeeping gene β-actin mRNA levels using the Livak method ($2^{-\Delta\Delta Ct}$) [32].

Table 1. Treatment details and PBMC stimulation in each group.

Group	Treatment
1	PBMC + Concanavalin-A 1 µg/mL (Positive Inflammation Control)
2	PBMC + DENV-1 + Antibody
3	PBMC + DENV-1 + Antibody + FVP 2xIC_{50}
4	PBMC + DENV-1 + Antibody + Quinine Sulfate 2xIC_{50}

Table 2. Primer sequences for cytokine gene analysis using real-time PCR.

No.	Primer	Orientation	Sequences (5′- 3′)	Size (bp)	Reference
1.	β-actin	Forward	AGAAAATCTGGCACCACACC	395	[33]
		Reverse	CTCCTTAATGTCACGCACGA		
2.	TNF-α	Forward	TGCTTGTTCCTCAGCCTCTT	186	[33]
		Reverse	ATGGGCTACAGGCTTGTCACT		
3.	NF-κB	Forward	ATCCCATCTTTGACAATCGTGC	153	[34]
		Reverse	CTGGTCCCGTGAAATACACCTC		
4.	IL-6	Forward	GTACATCCTCGACGGCATC	384	[33]
		Reverse	AGCCACTGGTTCTGTGCCT		
5.	IL-10	Forward	GCCTAACATGCTTCGAGATC	206	[35]
		Reverse	TGATGTCTGGGTCTTGGTTC		

Statistical analysis

Data analysis was performed using IBM SPSS Statistics Version 26 (IBM, SPSS Inc., New York, USA) and GraphPad Prism Version 10 statistical software (GraphPad Software, Inc., California, USA). Data were collected based on the IC_{50}, CC_{50}, and anti-inflammatory test results. IC_{50} and CC_{50} values were obtained using linear regression. After obtaining IC_{50} and CC_{50}, the Selectivity Index (SI) value can be determined using the ratio between IC_{50} and CC_{50}. In IC_{50} and CC_{50} values, data normality and homogeneity were analyzed using the Shapiro-Wilks Test. Normal data will then be subjected to the one-way ANOVA test to see the meaningfulness of the mean difference between the treatment group and the control group. Data that are not homogeneous can use non-parametric tests to determine the meaningfulness of the difference in means between the treatment group and the control group.

RESULTS

IC_{50} value of favipiravir

FFA was used to determine the titer of DENV after being treated with FVP. One foci on FFA represented 1 DENV that replicated during 48 h of the incubation time. Treatment with FVP decreased the number of foci in a dose-dependent manner (Figure 1). The control group treated with 0.1% DMSO showed a large number of DENV-1 foci. The Mann-Whitney test showed a significant difference between the number of foci at each concentration of FVP and 0.1% DMSO control with p-value ≤ 0.01.

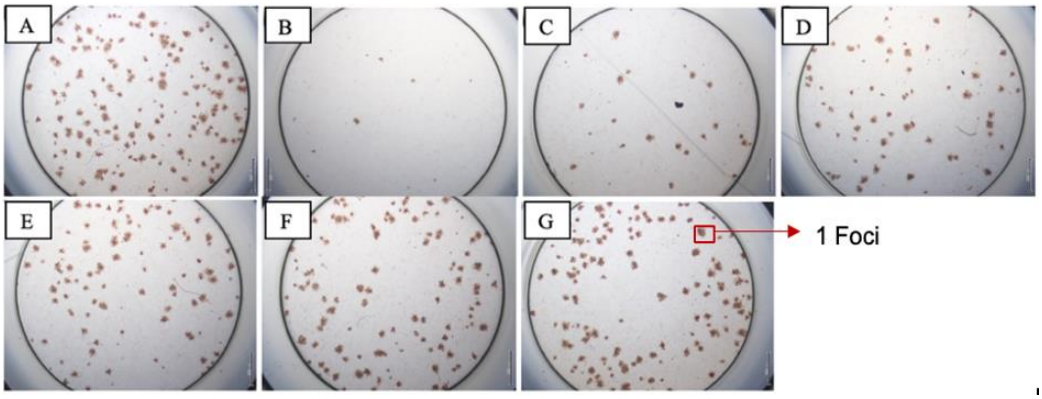

Figure 1. DENV-1 focus assay results in Vero E6 C1008 cells treated with various concentrations of Favipiravir. 1 foci came from one infected DENV-1 that was replicated in Vero cells for 2 days of incubation. A. Negative Control (DMSO 0.1%), B. Favipiravir 10 µg/mL, C. Favipiravir 5 µg/mL, D. Favipiravir 2.5 µg/mL, E. Favipiravir 1.25 µg/mL, F. Favipiravir 0.625 µg/mL, G. Favipiravir 0.3125 µg/mL. Using 20x magnification and Focus Forming Assay (FFA).

The percentage of inhibition was obtained by comparing the difference in the number of foci in the control group with the treatment group at each concentration then divided by the number of DENV-1 foci in the control group. The higher the concentration of FVP showed the higher the percentage of inhibition (Figure 2 and Table 3).

The percentage of inhibition and FVP concentrations were used to create a linear regression graph. The linear regression equation obtained was $y = 6.4725x + 32.264$ with R^2 of 0.9448 (Figure 2). From the above equation, the IC_{50} value of FVP was 2.72 µg/mL.

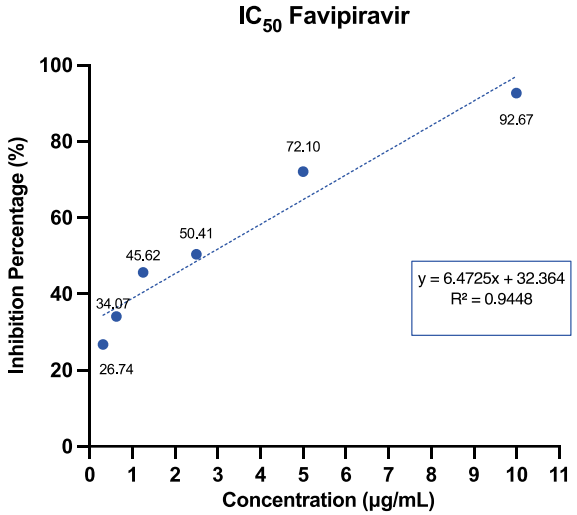

Figure 2. Linear regression graph between favipiravir concentration and percent inhibition. The R-value was 0.9448, indicating that experiments were in a dose-dependent manner.

Table 3. Number of DENV-1 foci by favipiravir at various concentrations.

Favipiravir concentration (µg/mL)	Number of foci treatment (FFU ± SD)*
10	8.67 ± 2.52[a]
5	33.00 ± 3.61[a]
2.5	58.67 ± 7.57[a]
1.25	64.33 ± 6.66[a]
0.625	78.00 ± 8.19[a]
0.3125	86.67 ± 1.53[a]
DMSO 0.1% Control	118.33 ± 8.07

* Performed by focus forming assay (immunostaining); a :*p value* ≤ 0.01. The mean number was counted from triplicate experiments.

IC_{50} value of quinine sulfate

The determining protocol of the IC_{50} value of QS is the same as FVP. Administration of QS decreased the number of DENV-1 foci in a dose-dependent manner (Figure 3 and Table 4). The control group treated with 0.1% DMSO showed the highest number of foci (Figure 3 and Table 4). After being treated with QS at a concentration of 160 ug/mL, no foci with brown color dots appeared. With the reduced concentration of QS, the number of foci increased (Figure 3 and Table 4).

Based on the logarithmic regression graph, a line equation was used to obtain the value of IC_{50}. The log regression was y = 34.751ln(x) – 44.412 with R^2 of 0.9313 (Figure 4). The IC_{50} value of QS was 14.97 µg/mL.

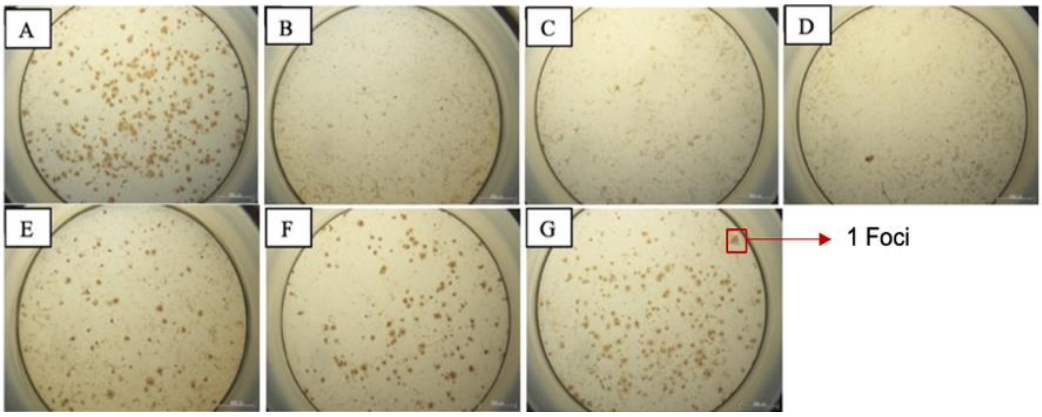

Figure 3. DENV-1 focus assay results in Vero E6 C1008 cells treated with various concentrations of Quinine Sulfate. One foci came from one infected DENV-1 that was replicated in Vero cells for 2 days of incubation. A. Negative Control (DMSO 0,1%), B. Quinine Sulfate 160 µg/mL, C. Quinine Sulfate 80 µg/mL, D. Quinine Sulfate 40 µg/mL, E. Quinine Sulfate 20 µg/mL, F. Quinine Sulfate 10 µg/mL, G. Quinine Sulfate 5 µg/mL. Using 20x magnification.

Table 4. Number of DENV-1 foci by quinine sulfate at various concentrations.

Quinine Sulfate concentration (µg/mL)	Mean number of foci treatment (FFU ± SD)*
160	0.00 ± 0.00[a]
80	0.00 ± 0.00[a]
40	2.33 ± 0.58[b]
20	72.33 ± 7.37[b]
10	131.33 ± 8.14[b]
5	145.67 ± 9.29[b]
0 (Control)	174.5 ± 9.73

* performed by focus forming assay (immunostaining) a: *p-value* ≤ 0.01; b: *p-value* ≤ 0.05. The mean number was counted from triplicate experiments.

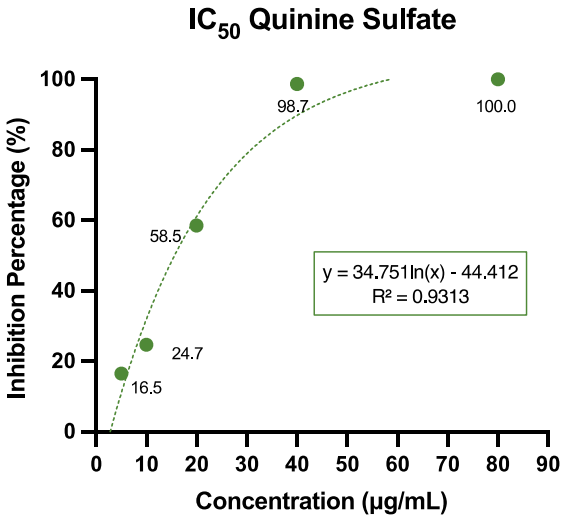

Figure 4. Logarithmic regression graph between Quinine Sulfate concentration and percent inhibition. The R-value was 0.9313, indicating that experiments were in a dose-dependent manner.

CC_{50} value of favipiravir and quinine sulfate

Based on the MTT activity, the increased FVP and QS concentration reduced the cell viability (Figures 5 and 6). FVP at a concentration of 160 µg/mL, the viability of the cells

was only 51 ± 3.3 %. At concentrations less than 10 µg/mL of FVP, it didn't show any toxicity to the cells, indicating that the higher the concentration of FVP, the more toxic to cells (Supplementary Table 1).

Determination of the CC_{50} value was done by creating a regression graph between the percentage of viability and FVP concentration (Figure 5). The linear regression equation of FVP treatment was y = -0.303x + 97.504 with R^2 = 0.9725. Based on the above equation, the CC_{50} value of FVP was 156.78 µg/mL.

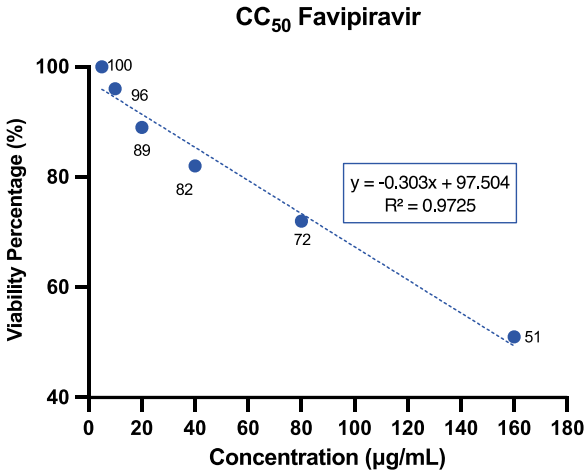

Figure 5. The percentage viability of Vero cells at various concentrations of favipiravir. The R-value was 0.9725, indicating that experiments were in a dose-dependent manner.

Based on the MTT activity, the increased QS concentration reduced the cell viability (Figure 6). QS at Concentrations of 10, 5, 2.5, and 1.25 µg/mL showed percentage cell viability significantly different from the 0.1% DMSO control group. Fortunately, the concentration of QS at 0.625 µg/mL was not significantly different (Supplementary Table 2).

The logarithmic regression equation of QS treatment was y = -9.506In(x) + 86.298 with R^2 = 0.9775 (Figure 6). Based on the above equation, the CC50 value of QS was 85.2 µg/mL.

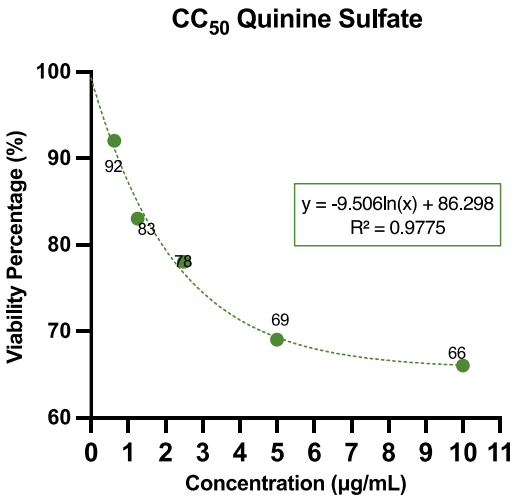

Figure 6. The percentage viability of Vero cells at various concentrations of quinine sulfate. The R-value was 0.9775, indicating that experiments were in a dose-dependent manner.

Selectivity index

The selectivity index (SI) value was obtained by comparing the CC_{50} and IC_{50} values of FVP and QS. From this study, the SI values of FVP and QS were 58 and 5.69, respectively (Table 5).

Table 5. SI values of FVP and QS. Calculated from the comparison of CC_{50} and IC_{50} values.

Drug	IC50 (μg/mL)	CC50 (μg/mL)	SI
FVP	2.72	156.78	58
QS	14.97	85.2	5.69

Anti-inflammatory activity of favipiravir and quinine sulfate

Anti-inflammatory activity was observed in the anti-DENV antibody present as an ADE model. PBMC treated with 0.1% DMSO was used as a negative control. Relative mRNA expression was analyzed against housekeeping gene β-actin mRNA levels using the Livak method ($2^{-\Delta\Delta Ct}$). Each relative mRNA expression value was represented from the triplicate sample. In comparison with 0.1% DMSO control, after 24 h of incubation, the expression of TNF-α, IL-6, IL-10, and NF-κB increased in all treated groups in PBMC (data not shown). In comparison with DENV-infected PBMC, expression of NF-κB was more profound in DENV-infected PBMC treated with QS (Figure 7A). Expression of TNF-α was slightly increased in DENV-infected PBMC treated with FVP and QS (Figure 7B). IL-6 and IL-10 cytokines expression decreased after being treated with FVP (Figure 7C and D). Similar to FVP, treatment with QS also decreased the expression of IL-6 and IL-10 cytokines (Figure 7C and D).

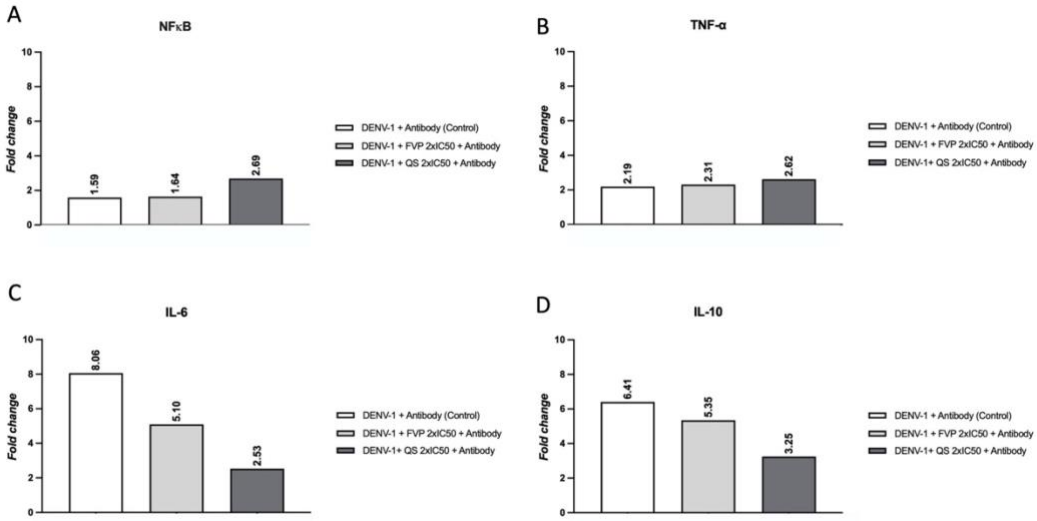

Figure 7. Relative mRNA expression of transcription factor A) NF-κB and cytokines B) TNF-α, C) IL-6, and D) IL10 after treatment with favipiravir and quinine sulfate in PBMC infected with DENV-1 in the presence of antibodies as ADE model. Each relative mRNA expression value was represented from a triplicate sample. The doses of FVP and QS used were $2xIC_{50}$, as determined by the antiviral activity test.

DISCUSSION

Up to now, there are no specific commercial antiviral drugs for DENV infection. Poorly coordinated clinical trial efforts, difficulties in animal models and laboratory tests, and complex serotypes and genotypes have hampered antiviral drug development. Several candidates for anti-DENV drugs that are direct-acting antiviral (DAA) and host-directed antiviral (HDA) have been explored, but none have been successful in effectively treating dengue infection [36, 37]. The discovery of new anti-DENV drugs takes a long time and involves many steps, and repurposing drugs is an alternative strategy to accelerate this process. In addition, repurposing drugs are already guaranteed to be safe [38]. There is an urgent need for the development of therapeutics against dengue. This study evaluated the potential antiviral and anti-inflammatory effects of FVP and QS in DENV-infected Vero cells and PBMC.

FVP inhibited DENV replication in HUH-7 cells and SK-N-MC cells with EC_{50} of 146.8 μM, 110 μM, and 287.9 μM. Besides FVP, QS also has the potential to have an antiviral effect on DENV with an EC_{50} of 33.31 μg/mL [23, 26, 39]. In the current study, the IC_{50} value was determined by FFA, therefore, the target of drug inhibition against virus replication was not determined. It found the IC_{50} of FVP and QS was 2.72 μg/mL and 14.97 μg/mL, respectively. This showed that FVP has a stronger DENV-1 antiviral potential than QS. FVP is a broad-spectrum antiviral drug and is widely used for the treatment of RNA virus infections, which targeting the RNA-dependent RNA polymerase (RdRp) leads to chain termination during viral replication and viral mutagenesis [22]. The mechanism of QS was increasing RIG-I expression and stimulating the interferon type I (IFN I) pathway to produce IFN-α. IFN-α then stimulates interferon-stimulated genes (ISRE) to induce RNase L and protein kinase R (PKR). Induction of both genes can inhibit the synthesis of viral proteins to inhibit the replication of the virus. Activation of PKR can block the viral translation process, and RNase L can degrade viral mRNA so that no viral RNA is replicated [24, 26].

FVP and QS were tested for toxicity against Vero cells to obtain a concentration that reduced the number of viable cells tested by 50%. The CC_{50} values of FVP and QS were 156.72 μg/mL and 85.2 μg/mL. In previous studies, the CC_{50} value of FVP on HUH-7 cells was > 500 μM or equivalent to 78.5 μg/mL [23] and > 1000 μM or equivalent to 157 μg/mL [39]. The CC_{50} of FVP was also evaluated using other cells such as MDCK cells, Vero cells, HEL cells, A549 cells, HeLa cells, and HEp-2 cells. The resulting CC_{50} value of FVP for all those cells was>1000 μg/mL or equivalent to 157 μg/mL, indicating that FVP was not toxic to the cells tested at concentrations up to 1000 μg/mL [40]. The FVP causes cell damage by oxidative stress, which can cause DNA damage in rat cardio myoblast cells (H9c2) and human skin fibroblasts (CCD-1079Sk). Oxidative stress is observed in the presence of ATP depletion, which is a sign of cellular stress [41]. Focusing on the QS drug, a previous study showed CC_{50} value of QS was 322.2 μM or 104.52 μg/mL [26].

A compound used as a drug candidate must have a high toxic concentration value and a very low activity concentration. DENV candidate antiviral with SI values ≥ 10 are generally considered to have potential as antiviral drug for further drug development [42, 43]. The SI values FVP and QS were 58 and 5.69, respectively. FVP has better potential to be anti-DENV-1 compared to QS. Previous studies using different cell lines showed that the SI value of FVP was 9.1 [39]. The SI results of QS were similar to previous research, where the SI value was 3.137 [26].

Besides the capability to inhibit DENV replication, candidate antivirals are also suggested to have potential as anti-inflammatory since the severe DENV caused by

cytokines storm. The present study showed that FVP and QS reduced the expression of IL-6 and IL-10 in DENV-infected PBMC with antibodies. Even though the main cytokine target (TNF-α) reduction did not occur after being treated with FVP and QS. FVP significantly reduced the levels of proinflammatory cytokines such as IL-6 and IL-10 in mice infected with Crimean-Congo hemorrhagic fever virus (CCHVF) [44]. Previous research found that QS has anti-inflammatory activity by inhibiting cytokine production and IL-6 release in T cells [45]. QS also suppressed the production of Th2-related cytokines such as IL-4, IL-5, IL-13, IL-1β, and TNF-α by inhibiting the activity of the NF-κB signaling pathway [46]. Quinine treatment effectively attenuated the infiltration of inflammatory cells, alleviating the pathological damage, inhibiting the expression of genes related to the NF-κB signaling pathway, and reducing the inflammatory response [46]. In COVID-19 infection, the production of the cytokine TNF-α is also suppressed [45, 47]. In addition, quinine effectively enhances the production of IFN-α cytokine, which acts as an immune stimulator to inhibit the virus [47]. In contrast, we found that the NF-κB activity and TNF-α suppression did not occur. It may be due to the concentration and incubation time for 24 h of FVP and QS as well as the influence of cytokine kinetics.

However, this study has several limitations. Specifically, there was no examination of the amount of viral RNA in the cells, the number of infective virions, or the number of cytokines secreted in the supernatant of PBMC in the anti-inflammatory test. Furthermore, the anti-inflammatory activity test has not been repeated, which precludes statistical analysis.

CONCLUSIONS

In conclusion, FVP has potential as an antiviral to DENV-1 and reduced IL-6 and IL-10 cytokines expression in DENV-infected PBMC. However, in comparison with QS, FVP showed lower anti-inflammatory activities. Further studies are needed to explore the mechanisms by which both repurposing drugs inhibit DENV replication and mediate anti-inflammatory effects.

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AUTHOR CONTRIBUTIONS

All authors contributed to the development of this paper. RAR: data curation, formal analysis, investigation, methodology, software, visualization, writing - original draft, writing – review, and editing. BED and FIT: Conceptualization, formal analysis, investigation, methodology, writing - review and editing. All authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

SUPPLEMENTARY MATERIALS

Supplementary Table 1. Percentage of Vero cell viability after being treated with various concentrations of Favipiravir, and Supplementary Table 2. Percentage of Vero cell viability after being treated with various concentrations of quinine sulfate (Supplementary Materials).

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