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SAUCHINONE WITH ZINC SULPHATE SIGNIFICANTLY INHIBITS THE ACTIVITY OF SARS-COV-2 3CL-PROTEASE

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Abstract

Coronavirus-2019 (COVID-2019), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been declared a pandemic due to its global threats to public health and resulting economic losses. Currently, no specific and effective antivirals for SARS-CoV-2 infection exist. Despite the development of vaccines, the high transformation rate of the COVID-19 infection have dictated that an finding an effective treatment is urgent. The 3CL protease plays an essential role in viral replication; therefore, it is recognized as an attractive drug discovery target. In this study, we investigated sauchinone and the combination of sauchinone/Zn-II as potential inhibitory agents against 3CL-Protease activity. The 3CL Protease Assay Kit was used to determine 3CL protease activity. A commercial tetrazolium MTS assay kit was used to determine cytotoxicity. Sauchinone produced a dose-dependent inhibitory effect on 3CL protease with a half inhibitory concentration (IC50) of 4.325 µM. Zinc sulphate monohydrate (2 mg/mL) demonstrated an additive and inhibitory effect of 2.02-fold when used in combination with sauchinone against 3CL-protease activity when compared with the effects of 5 µM sauchinone against 3CL-protease activity with a significant inhibitory activity ($p < 0.001$). Cytotoxicity was not observed for either 30 µM sauchione or sauchinone (30 µM)/Zn-II (4 mg/mL). Our findings may provide important information for the optimization and design of more potent inhibitors against the 3CL-protease to be used as potential antiviral agents against COVID-19.

Keywords: *SARS-CoV-2 3CL-Protease, Sauchinone, Zinc-II, Antiviral against COVID-2019.*

Introduction

Coronavirus-2019 (COVID 19), caused by the severe acute respiratory syndrome coronavirus 2, started in 2019 in Wuhan, China, and after escalation of this disease, the World Health Organization declared this disease a pandemic. The disease has spread worldwide causing more than 171 million infected people and more than 3.5 million deaths, in addition to causing huge worldwide economic worldwide (1).

All of the currently available drugs, including remdesivir, have low clinical efficacy or must be used in the early stages of the disease. Also, the concept of relying on herd immunity to cause the pandemic to recede may be disappointing and fruitless. Although effective vaccines are available, the high transformation rate of the COVID-19 infection is likely to make it necessary to receive annual vaccinations, which consumes time and money; therefore, the discovery effective treatments is necessary (2). SARS-CoV-2 3CL-protease (3CL-protease) plays a vital role in processing the translation of polyproteins from viral RNA. The inhibitors of 3CL protease can block virus replication and could be potential drug candidates for the treatment of coronavirus infections (3–5).

Sauchinone is an anti-inflammatory and antioxidant lignan isolated from Saururus chinensis. S. chinensis is an herb used in Oriental folk medicine to treat fever, jaundice, edema, and inflammatory diseases. Sauchinone inhibits lipopolysaccharide (LPS)-induced macrophage expression of inducible nitric oxide synthase, tumor necrosis factor alpha, and cyclo-oxygenase 2 (iNOS/NOS II, TNF-α, and COX-2, respectively) by inhibiting the inhibitor of kB (IKBα) phosphorylation. It may be possible to use sauchinone to prevent myocardial ischemia/reperfusion injury via inhibition of p28 phosphorylation (6–8). Zinc II has antiviral activity because it has the capability to inhibit both proteolytic and polymerase enzymes. Recently, the combination of punicalagin with zinc II was shown to be strongly inhibitory against 3CL-protease activity (9).

Therefore, this study aimed to determine the inhibitory effects of sauchinone and the combination of sauchinone with Zn II against 3CL-Protease activity.

Methods

Materials

Zinc sulfate monohydrate and sauchinone ≥ 98% (high-performance liquid chromatography grade [HPLC]) were purchased from Sigma Aldrich (Saint Louis, MO).

3CL protease assay protocol

Improved 3CL Protease Assay Kit (BPS Bioscience, #78042, San Diego, USA) was used to assess the effect of sauchinone, Zn-II, and sauchinone /Zn-II on the activity of the 3CL protease. A Tecan microplate fluorimeter (Tecan Biotek, Winooski, VT) equipped with excitation and emission at 360 and 460 nm, respectively, was used to estimate fluorescence (9–11). according to the manufacturer's instructions.

Cytotoxicity

Sauchinone-induced cytotoxicity was analyzed using the Cell Titer 96 Aqueous Kit (Promega, Southampton, UK) as previously described (9,12,13).

Statistical analysis

The GraphPad Prism 9 and SPSS 19.0 software for data analyses were used. Differences between study groups were determined based on a one-way analysis of variance (ANOVA) and Tukey's multiple comparisons as a post-hoc test. $P < 0.05$ was considered statistically significant.

Results

Inhibitory effect of sauchinone on 3CL protease activity

A decrease in the activity of the 3CL-protease was observed with an increase in the sauchinone concentration as shown in Figure 1.

Therefore, the half maximal inhibitory concentration (IC50) of sauchinone was calculated and was equal to 4.325 µM (Figure 2). From Figures 1 and 2, the continuation of the decrease in the activity of the 3CL-protease can be seen until a concentration of 5 µM was reached. After this point, the decrease in the activity of the enzyme became minimal and barely changed, so a concentration of 5 µM was selected.

The inhibitory effect of sauchinone/Zn-II on 3CL Protease Activity

When studying the activity of 3CL Protease using a combination of 5 µM sauchinone with

different concentrations of zinc sulfate monohydrate (0, 0.2, 0.5, 1, 1.5, 2, 3, 5, 20, 40, and 50 mg/mL), a greater decrease in 3CL Protease activity was observed with a combination of sauchinone and zinc sulfate when compared with sauchinone only. It was observed that the concentration that yielded the best maximum decrease in the activity of the 3CL Protease was sauchinone (5 µM) with Zn sulfate monohydrate (2 mg/mL) as shown in Figure 3.

The coefficient of drug interaction (CDI) as measured for 3CL protease activity with sauchinone (5 µM)/Zn sulfate monohydrate (2 mg/mL) was 1.23, indicating that sauchinone/Zn-II has an additive rather than synergistic effect (Figure 4). For sauchinone/Zn-II compared to sauchinone (5 µM) alone 3CL protease activity was reduced by 2.02-fold, as shown in Figure 5.

Cytotoxicity

No cytotoxic effects based on the tetrazolium MTS proliferation assay were seen after using sauchinone at 30 µM, Zn sulfate monohydrate at 4 mg/mL, and a combination of the two agents at these concentrations with no significant differences between any combination ($p > 0.05$) at 0, 24, 48, and 72 h between application of formulations. This finding indicates that these drugs do not affect the percentage of viable cells after they are applied (Figure 6).

Discussion

3CL protease is the main protease of the coronavirus (14). This enzyme is used for proteolytic processing during virus maturation. The functional importance of this proteolytic enzyme in the life cycle of the virus points to the 3CL protease as a promising target for the development of anti-SARS-CoV-2 drugs (15).

Sauchinone is a natural drug with known antiviral effects. We found powerful inhibitory activity of sauchinone against 3CL protease with highly significant inhibition against its proteolytic activity ($P < 0.001$) without any cytotoxicity. The IC50 of sauchinone was 4.325 µM. GRL-1720 (indolin moiety), 5h (indole moiety), and GC376 also potently inhibited 3CL-protease, thus preventing infection caused by SARS-CoV-2. These agents also prevented cellulopathy and infection at high efficiencies (2). Moreover, phenolic and polyphenol compounds, such as punicalagin, theaflavin, and epigallocathechin (EGCG), generated dose-dependent inhibitory effects on the 3CL protease. The IC50 values were 6.192, 8.44, and 7.58 µg/ml for punicalagin, theaflavin, and EGCG, respectively (9,16). Also, peptidomimetic α-ketoamides represent prototypical inhibitors of 3CL-protease (15).

The most reasonable explanation for these result could be that the active site of the 3CL protease contains many highly conserved substrate binding sites (17). Therefore, this structure allows for the presence of many and varied designed or natural compounds that inhibit proteases.

Likewise, late molecular docking studies have confirmed that sauchinone may be useful as an inhibitor of the 3CL-protease because it shows excellent binding affinities in very stable complexes with 3CL-protease. Sauchinone interacts with active site residues. It forms two conventional hydrogen bonds with M49 and G143 and a carbon-hydrogen bond with N142. In addition, the 3CL-protease-sauchinone complex also shows van der Waals interactions with R188, D187, P52, C44, H41, T25, L141, E166, and Q189, hydrophobic interactions between alkyl groups and C145 and M165, hydrophobic interaction of Pi-alkyl groups with C145 and M165 (Molecular Mechanics Poisson-Boltzmann surface area [MMPBSA]). The MMPBSA results showed that the sauchinone and the 3CL-protease formed a very stable complex with a free energy of −71.68 kJ mol-1 (18).

Zinc is capable of inhibiting proteolytic and polymerase enzymes since it has antiviral properties 17,18; however, we found that zinc inhibited 3CL-protease but not significantly ($P > 0.05$).However, the Zinc-II produced an additive effect when combined with sauchinone, leading to 2.02-fold greater decrease in the activity of 3CL-protease compare with sauchinone only. Zinc-II may bind to the active site of 3CL protease and achieve coordination with the active site. Similar studies show synergistic effects of Zn-II when combined with punicalagin with extremely strong inhibition of 3CL-protease activity ($p < 0.001$) (9). Both pomegranate peel extract (PRE) and punicalagin when co-administered with zinc (II) ions have enhanced viricidal activity against herpes simplex virus (HSV) (19-21).

In conclusion, Inhibition of the SARS-CoV-2 3CL-protease is a promising strategy for drug discovery. In this study, the natural product, sauchinone, combined with Zn-II appears to be a potential inhibitor of 3CL-protease in vitro. These observations suggest that this combination of sauchinone/Zn-II could be used as suitable candidate drug for the treatment of COVID-19 without causing any cytotoxicity. This mixture makes it difficult for the virus to adapt to the host cell, which helps the host limit resistance to infection and is also not affected by viral mutations.

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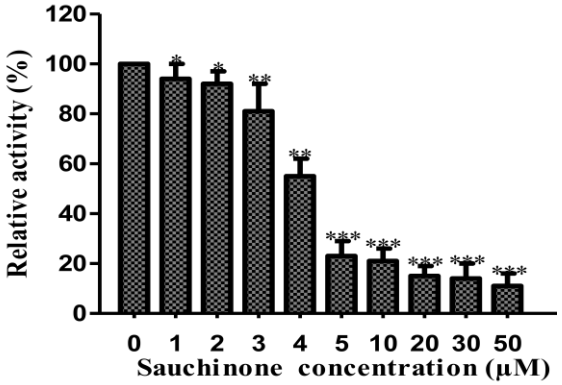

FIGURE 1. Effect of different concentrations of sauchinone on 3CL-protease activity in vitro. (mean ± standard deviation [SD], analyzed in triplicate). *P > 0.05, **P < 0.01, ***P < 0.001.

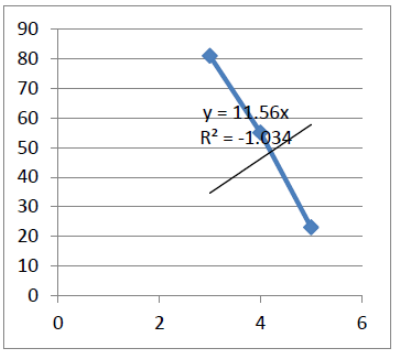

FIGURE 2. The half maximal inhibitory concentration (IC50) of sauchinone.

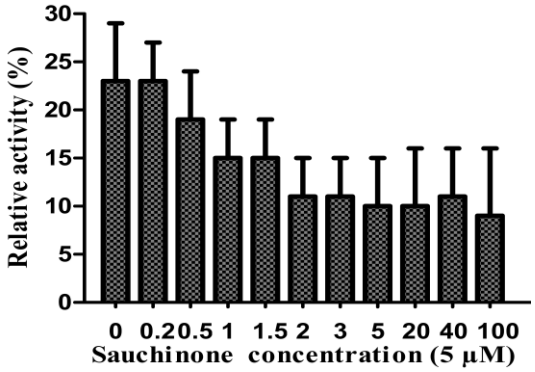

FIGURE 3. The impact of 5 μM sauchinone with different concentrations of zinc sulfate monohydrate on 3CL-protease activity (mean ± SD, analyzed in triplicate).

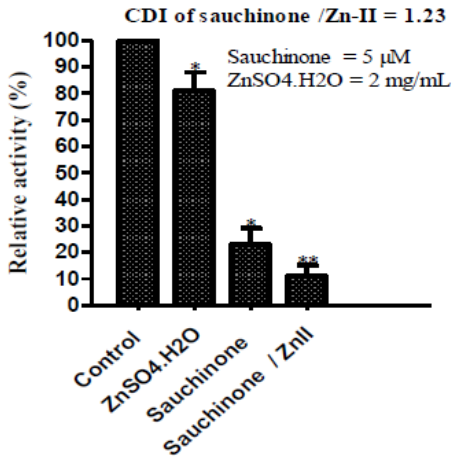

FIGURE 4. The inhibitory effect of sauchinone, ZnSO4.H2O, and sauchinone/Zn-II. The coefficient of drug interaction (CDI) was determined. *P < 0.01, **P < 0.001.

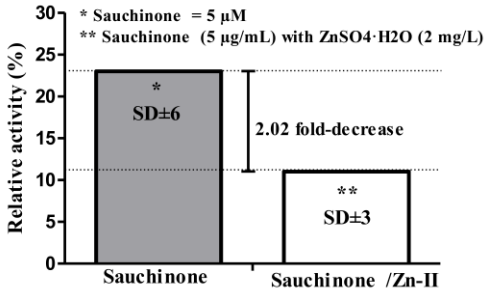

FIGURE 5. Comparison of the fold-decrease of 3CL-protease activity for sauchinone and sauchinone/ZnII.

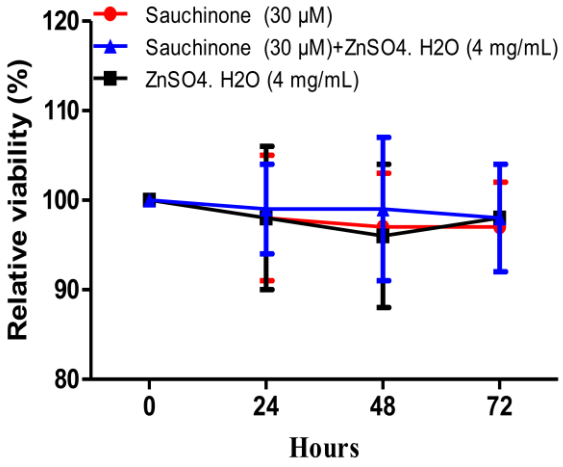

FIGURE 6. Cytotoxic effects of (1) sauchinone (30 µM), (2) ZnSO4. H2O (4 mg/mL), (3) combination of sauchinone (30 µM) + ZnSO4. H2O (4 mg/mL). No cytotoxic effects were observed at the concentrations used in this study.