Pharmaceutical Sciences And Biomedical Analysis Journal

SCIENTIFIC LITERATURE

Research Article

Topoisomerase II Inhibitor as a Potential Therapy for Severe COVID-19: Antiviral Activity and Molecular Docking Studies

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ARTICLE INFO

Received Date: August 30, 2022
Accepted Date: September 24, 2022
Published Date: September 29, 2022

KEYWORDS

SARS-CoV-2; Etoposide;
Antiviral activity; Crystal violet assay
Molecular docking; Etoposide

Citation for this article: Mahmoud M. Sebaiy, Mohamed Y. Hassaballah and Noha I. Ziedan. Topoisomerase II Inhibitor as a Potential Therapy for Severe COVID-19: Antiviral Activity and Molecular Docking Studies. Pharmaceutical Sciences And Biomedical Analysis Journal. 2022; 4(2):132

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ABSTRACT

Aim: SARS-CoV-2 has caused the current outbreak of the novel coronavirus pandemic (nCOV-19) and infected more than 42 million individuals worldwide. Currently, there is an urgent need to find potential drugs that can treat SARS-CoV-2 infection.

Background: Etoposide was suggested as a potential rescue treatment of severe COVID-19 cases since it possesses anti-inflammatory activity synergizing other therapies, including irinotecan and dexamethasone.

Objective: Herein, we discuss the potential subtle antiviral activity and possible viral targets of etoposide.

Main Method: The antiviral activity was tested by crystal violet assay. Molecular docking was carried out to determine the probable viral target.

Key findings: The results revealed inhibition of infected Vero E6 cells with IC_{50}= 21.30 µM, while cytotoxicity was CC_{50}>100 µM. Molecular docking study showed that Etoposide had a plausible binding affinity for RdRp and 3CLpro, which are two essential viral proteins for the SARS-COV-2 replication.

Significance: The findings suggest that Etoposide is promising as a candidate for the COVID-19 treatment that can be further tested for its efficacy *in-vivo* and further validation through clinical trials.

INTRODUCTION

The recent coronavirus pandemic (COVID-19) has infected 215 countries worldwide. Since the outbreak, enormous drug repurposing has been the logical solution by examining FDA-approved drugs; however, there are still no approved therapies or reliable preventative medications for COVID-19 [1]. There is enough evidence advocating the repurposing of Etoposide (ETP) as a treatment for COVID-19. Several drugs that have been approved for a cancer indication by the US FDA are now in COVID-19 clinical trials [2]; ETP was among these repurposed FDA approved drugs. Molecular docking of ETP using AutodockVina had shown the potential for 3CLpro inhibition [3,4].

ETP is a chemotherapeutic agent acting as a topoisomerase II enzyme inhibitor [5]. Anticancer medications are less encouraged for the treatment of COVID-19 because of the possibility of developing secondary malignancy;however, the estimated risk forETP was in the range of (0.3%-0.4%) [6]. Severe COVID-19 cases were accompanied by severe acute respiratory distress syndrome, explained by the

cytokine storm [7,8]. The monocytes-macrophages path stimulation has been described in patients with COVID-19 [9,10]. In SARS-CoVinfected mice, inflammatory monocyte-macrophage responses were related to lethal pneumonia, suggesting the importance of suppressing the monocyte-macrophage system in treating severe pneumonia associated with SARS-CoV [11]. Bronchoalveolar fluid single-cell analysis, especially in severe COVID-19 cases, has shown increased proportions of phagocytes [12]. These macrophages can also promote fibrosis generation. Additionally, a significant increase of $CD14^{+}CD16^{+}$ monocytes was also detected in patients with severe COVID-19 [13].

Furthermore, ETP was reported to damage antiviral triggered T cells approximately 100-fold, while the naive and memory T cells remained unaffected, thus indirectly reducing inflammatory cytokine levels [14]. It was also documented to suppress cytokine storm associated with Hemophagocytic Lymphohistiocytosis (HLH) [12,15] and was included in the HLH-94 protocol that is Etoposide-based treatment course, including dexamethasone for secondary HLH. Also, the recent findings on Dexamethasone clinical trials suggest a clinical benefit for the subset of patients requiring oxygen supplementation and those in the ICU with a lack of benefit for the subgroup of patients with mild and moderate infections [16,17]. Besides, in previous studies, the introduction of ETP in the early stage of EBV and H1N1 infection accompanied by sHLH and respiratory failure was largely effective [18]. These observations prompted the consideration of potential effectiveness of this cytotoxic therapy in HLH associated with moderately severe to severe forms of COVID-19 [19,20]. In light of this, ETP was suggested in a treatment protocol involving pre-administration of irinotecan, which is supposed to attenuate innate-adaptive imbalance-induced lymphocytopenia, thus creating a safe therapeutic window for ETP. Irinotecan and ETP were believed to behave synergistically via the attenuation of the cytokine storm while maintaining cell viability and minimizing host toxicity for critically ill COVID-19 patients [21].

It was reported that a female with COVID-19-associated hyperinflammatory state and hypoxic respiratory failure accompanied by severe cytokine storm was treated with dexamethasone followed by 50 mg/m^2 IV ETP once a week. The patient showed significant improvement in the inflammatory markers and oxygenation state and was permitted to quit ICU after five days [22]. Therefore, ETP has an additional anti-inflammatory effect mediated via the suppression of the monocytes-macrophages system hyperactivation caused by the inflammation caused by IL-6 and tackling the pulmonary fibrosis [12]. Low-dose ETP was found to improvehypercytokinemia and renew the cytotoxic T lymphocytes, thus eliminating the activated macrophages and SARS-CoV-2-infected cells [22]. Based on the stated facts, the possibility of using ETP as a part of the therapeutic strategy for COVID-19 patients was introduced. ETP was first repurposed to treat COVID-19 in a randomized controlled trial (NCT04356690) licensed for its ability to inhibit cytokine release syndrome. Herein, in addition to the prementioned anti-inflammatory effect, we highlight that ETO could have a subtle antiviral efficacy against the SARS-COV-2 virus.

MATERIAL AND METHODS

Material

Etoposide was supplied by Jiangsu Hengrui Medicine. DMEM and crystal violet were purchased from Sigma-Aldrich. DMSO, Methanol, and formaldehyde were of lab-grade from Thermo scientific. The SARS-COV-2 strain was (GSAID accession number: EPI_ISL_430820).

CC50 and IC50 Calculation using Crystal Violet Assay

The assay was performed according to the procedure that was previously described by Feoktistovaet al. [23] with minor modifications. Vero E6 cells were seeded into 96-well plates in 100 µl of DMEM Complete Medium containing DMEM high glucose medium with 2 mM L-glutamine, 1 mM sodium pyruvate, and 10% Fetal Bovine Serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin. After 24 h (90-100% confluence monolayer of Vero E6), each compound was diluted using infection DMEM into varying concentrations in a separate U shape 96 well plate (with a range of concentration from 10 µg/ml to 1 ng/ml). An aliquot of 100 µl of each dilution was transferred into new U shape 96 well plate and supplemented with 100 TCID50 in 100 µl infection media. In parallel, the wells dedicated for CC50 calculation were supplemented with 100 µl infection media without virus. Aliquots of 100 µl infection media containing 100 TCID50 were used as virus control. After 1 h of incubation, 100 µl of each well were transferred to their corresponding wells into the 96-well plates

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containing Vero E6 cultures (Figure 1). The plates were incubated for 72 h, and the cell monolayers were washed with PBS and subjected to cell fixation using 100 µl of 10% formaldehyde for 1 h. Subsequently, the plates are washed well for 3 times with 1x PBS and dried well before staining with 50 µl (0.5%) crystal violet to each well ((0.5 g crystal violet powder (Sigma-Aldrich), 80 mL distilled H_2O, and 20 mL methanol)) for 30 min. the plates were then washed well with rinsed water and air-dried at room temperature for 2-24 h. To distain crystal violet, 200 µl methanol were added to each well, and the plate was incubated with its lid on a bench rocker (20 oscillations/ minute) for 20 minutes at room temperature. Finally, the optical density of each well at 590 nm (OD590) was measured with a plate reader. 100% was assigned to non-treated control cells, the average OD of each dilution without or with the virus was compared to control cells and control virus wells to calculate % toxicity and % reduction in virus replication (respectively). The CC50 and IC50 values were calculated using non-linear regression (three parameters) in GraphPad Prism 5.01.

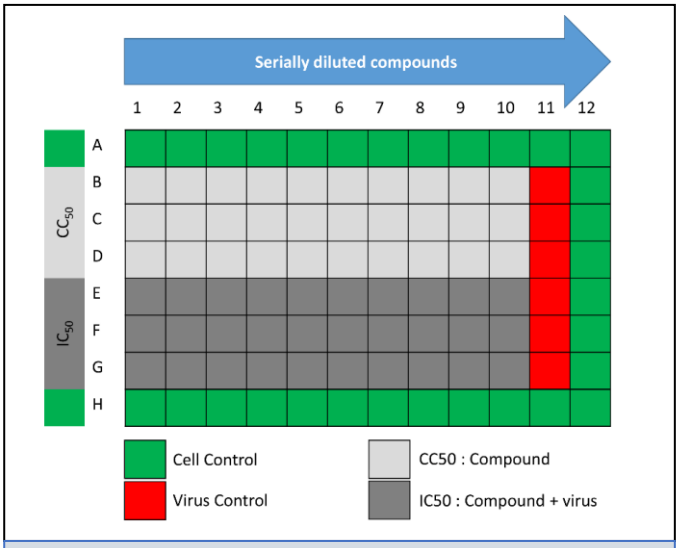

Figure 1: The schematic representation of the 96-well plate showing the serial dilution used in the crystal violet assay for determining the IC_{50} and CC_{50}.

Molecular Docking

2D structure of ETP was drawn using ChemDraw Ultra7.0 and exported to MOE 2019.01 software. The energy was minimized,applying 0.01 RMSD kcal/mol and Abmer10:EHT force field. Three viral target proteins, including RdRp (PDB ID: 7BV2), 3CL^{pro}(PDB ID: 6Y2G),and RBD-ACE-2 complex (PDB ID: 7C8D), were prepared first and protonated usingForcefield Amber10: EHT. ETP was docked using triangular matcher placement and London dG score while keeping receptor atoms rigid [24].

RESULTS AND DISCUSSION

Antiviral Activity of ETP

To determine whether inhibition of SARS-CoV-2 by ETP was cell-specific or not, we measured the half-maximum inhibitory concentration IC_{50} of ETP in SARS-COV-2 infected Vero E6 cell lines. In the present study, we noticed that ETP could inhibit SARS-CoV-2 strains proliferation during the early stages of viral infection. IC_{50}, as an estimation of the ETP viral inhibitory effect and CC_{50} as a cell viability testing, were obtained by non-linear regression analysis from the dose-response curve of ETP. It can inhibit the viral RNA replication at low concentration as its IC_{50} was found to be 21.30 µM, as seen in Figure 2 (A). Also, The cytotoxicity of ETP was tested in the same cells. The 50% cytotoxic concentration (CC_{50}) was estimated by linear regression of ETP concentration that reduces the optical density by 50% compared to the cell control. The results revealed that ETP has no remarkable toxicity at a high concentration where its CC_{50} was calculated to be 166.59 µM in targeted cells, as depicted in Figure 2 (B). These results indicate that ETP has a fair inhibitory activity against SARS-CoV-2 and an acceptable selectivity index (SI = CC_{50}/IC_{50}) value of 7.82. All these findings suggest that ETP can be potentially developed into an additional antiviral drug for the treatment of SARS-CoV-2 infections without exerting its typical side effects.

Molecular Docking

To find out why ETP shows antiviral properties against SARS-CoV-2, docking was done against three crucial viral targets, including RNA-Dependent RNA Polymerase (RdRp) that plays a role in the genetic material propagation (24, 25), 3-Chemotrypsin-Like protease (3CL^{pro}), which plays an important role in the viral replication process [26] and Receptor Binding Domain (RBD), which is important for the attachment step for the viral entry with the cellular ACE receptor [27].

The SARS-CoV-2 RdRp interaction with ETP was investigated. ETP has shown an appropriate affinity to the RdRp enzyme, which equals (-10.29) kcal/mol with a Root-Mean-Square Deviation (RSMD) of 1.96 Å. This binding score shows a relatively close affinity to the crystal ligand, which is (-11.05)

kcal/mol. The binding mode is shown in Figure 3, displaying two types of interactions between ETP and RdRp, including two electrostatic and three hydrogen-bonding interactions forming two H-bonds with A19 and one H-bond Arg*555*. The electrostatic interaction was formed between the ether oxygen and the hydroxyl group of cyclohexane moiety MG1005 and MG1004.

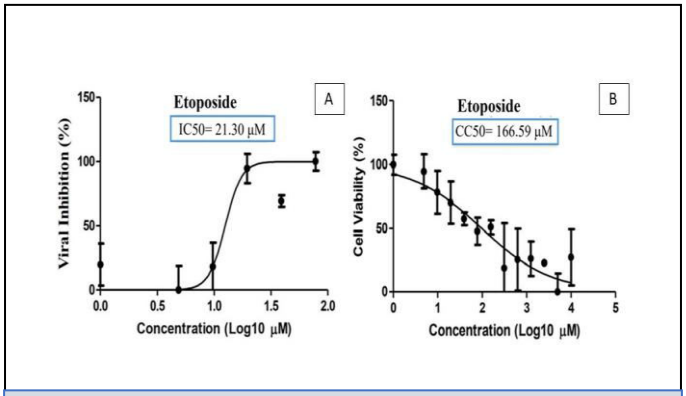

Figure 2: (A) IC_{50} of ETP in SARS-COV-2 infected Vero E6 cell lines. CPE induced by the virus in the presence and absence of drug was measured and expressed as percentage virus inhibition (percentage of drug-free control). ETP was added 5 min before virus infection and remained on the cells for the entire assay duration (72 h). IC_{50} was determined to be 21.30µM.(B) CC_{50} of ETPin SARS-COV-2 infected Vero E6 cell lines. Monolayers of Vero E6cellswere used to determine the cellular toxicity of ETP. The half-maximal response corresponding to the CC50 concentration of ETP was determined to be 166.59µM. All data shown are the average of 3 replicates ± SD.

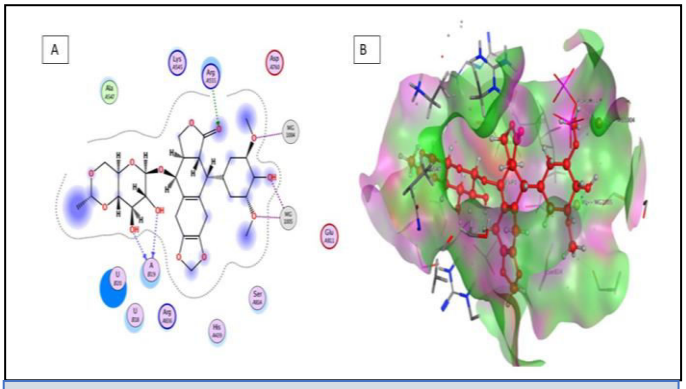

Figure 3: A) 2D binding mode representation of the binding site of RdRp with ETP. B) 3D representation of essential amino acid residue of RdRp binding with ETP.

The ETP binding to the catalytic site of SARS-CoV-2 3CLprowas displayed in Figure 4. ETP docking showed an affinity to $3CL^{pro}$ enzyme with a score of -7.98 kcal/mol compared to the crystal ligand score (-9.04). ETP also has a Root-Mean-Square Deviation (RSMD) of 1.78 Å lower than the ligand RMSD (2.22).The best pose is provided below in Figure 4, in which ETP formed two H-bonds with Met165 and Cys145 and hydrophobic interaction with His41.

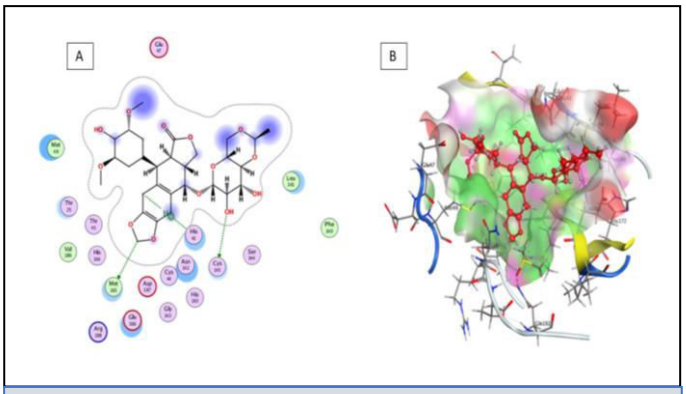

Figure 4: A)2D binding mode representation of the binding site of SARS-CoV-2 $3CL^{pro}$ with ETP. B) 3D representation of essential amino acid residue of SARS-CoV-2 $3CL^{pro}$ binding with ETP.

The Cryo-EM structure of cat ACE2 and SARS-CoV-2 RBD was used to identify the protein-protein interaction site and docking ETP at the active site. ETP was found to have no significant interaction with RBD.

CONCLUSION

Eoptoside possesses a proven activity in combating the inflammatory activity that has a potential role in SARS-CoV-2 infection progress to severe forms.The revealed antiviral activity in this study can be explained with docking results suggesting that RdRp and $3CL^{pro}$ are presumably viral targets for Etoposide. Etoposide is encouraging as a candidate for the COVID-19 treatment that can be further tested for its efficacy *in-vivo* and further validation through clinical trials.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available within the article.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared None

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