The Effect of SIRT1 activation on Cyclooxygenase enzyme activity in COVID-19 patients

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Abstract
For the third year COVID-19 pandemic is still a global health challenge, despite the availability of vaccines and protection methods, treatment protocols still being updated continuously to observe the optimum management for patients. Cyclooxegynase (COX) enzymes are involved in inflammation and thrombosis related to COVID-19. COX-Thromboxane2 pathway is one of the important pathways that results in thrombus formation. In this study the COX activity level changes were measured by ELISA technique in COVID-19 plasma samples that treated with SIRT1 activators resveratrol and linear BAS SIRT1 aptamer, a significant lowering in COX activity was observed with promising potential atrithrombotic action in COVID-19 to be further investigated in future.
Keywords: COVID-19, COVID-19 associated coagulopathy, Thrombosis, Cyclooxegenase, SIRT1

1. Introduction

Coagulation of blood is an essential process preventing blood loss due to vessels damage wounds or any other abnormal cause of blood loss, this physiological role is known as haemostasis and pathologically as thrombosis(1). Coagulation cascade and platelet aggregation (that start initially) coordinate together to obtain the hemostatic plug from fibrin and platelets(2). Platelets activation and aggregation starts initially (along with coagulation cascade) results eventually in thrombosis (3). This activation \ aggregation pathway include many factors, cyclooxygenase-1 (COX-1) enzyme is one of the important factors. Arachidonic acid is converted to cyclic endoperoxide prostaglandin (PG) G2 and H2 via cyclooxygenase 1 enzyme (COX-1), PGG2, and PGH2 are then converted to thromboxane A2 (TXA2) by TXA synthase (3,4). TXA2 acts through TXA2 receptor (TXA2R) which is G protein-coupled receptor (GPCR) resulting in morphological changes and platelet aggregation (and thrombus formation) through Gq-phospholipase C–inositol 1,4,5-trisphosphate– Ca2+ and G12\13 signaling pathways (5,6).

Coronavirus disease 2019 (COVID-19) is a respiratory disease, SARS-CoV-2 virus infection is the cause of the disease (7). Acute Respiratory Distress Syndrome (ARDS) is the most important COVID-19 complications (8), Hypercoagulation is involved in ARDS pathophysiology (9).

COVID-19 can cause many cardiovascular disease (CVD) complications. Patients who already have CVD tend to suffer from higher severity than regular patients (10). High morbidity and mortality of COVID-19 are linked to thrombotic complications. Thrombotic events related to CVD like myocardial infarction was reported in COVID-19 patients with severe illness, other thrombotic events (not related to CVD) like; Pulmonary embolism (PE), deep vein thrombosis (DVT), and ischemic stroke also has been reported in COVID-19 patients (11). Up to 30% of intensive care unit (ICU) admission patients suffered from thrombotic events (12).

Cyclooxygenase (COX) enzymes are involved in COVID-19 pathophysiology, COX-2 overexpression is linked to higher morbidity because of its inflammatory role. On other hand COX-1 has an important role in thrombosis (13, 3).

Silent information regulator 2 (known as Sirtuins) are a enzymes of nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases (14). Many Sirtuins have been discovered, SIRT1 is the most studied one (15).

SIRT1 activation has antithrombotic effect and SIRT1 activators were tested against COVID-19 associated coagulopathy with promising potential antithrombotic role (16).

Resveratrol is the first and most investigated SIRT1 activator, it is founded naturally in grape and red wine (17,18). Linear BAS SIRT1 aptamer is a novel SIRT1 ligand (DNA sequences consist of 40 nucleotidestheat bind to SIRT1 enzyme and modulate its activity within cells improving its enzymaric activity. Linear BAS SIRT1 aptamer is proved to be more potent, safe and effective SIRT1 activator among the other activators like resveratrol (19).

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2. Materials and methods

Materials: Linear BAS aptamer that activate SIRT1 was collected from BioNeer Company-Korea. Resveratrol and all chemicals were purchased from Sigma, USA. Cyclooxygenase activity assay (Fluorometric) kit was purchased from Elabscinces, USA.

Human subjects: The donators that gave blood samples for the study healthy non-smoking adults group (n = 10). They had no personal or family history of cancer, bleeding, and cardiovascular diseases or other diseases. Regarding COVID-19 samples group, patients ill with COVID-19 infection (n = 80) were recruited from Al-Shifaa Medical Center and Al-Kindi teaching hospital in Baghdad (capital of Iraq). COVID-19 infection was confirmed by reverse transcription polymerase chain reaction (RT-PCR). Anti-coagulated whole blood was collected from hospitalized COVID-19 patients from 11 November 2020 to 22 February 2021. All COVID-19 patients were recruited with the approved study protocols by the institutional review board (IRB) of the Al-Shifaa Medical Center and Al-Kindi teaching hospital in Baghdad's capital-Iraq. All patients enrolled withn 72 hours of hospitalization or intensive care unit (ICU) admission. Healthy, age and sex-matched donors were enrolled under a separate of institutional review board (IRB) protocol.

Blood samples and Platelet isolation

3.2% sodium citrate tube (pH 5.5 and 9:1 v/v ratio) were used for collection of peripheral venous blood drawing through standard venipuncture technique. immediately following the blood draw, Complete blood count and other hematological tests were performed.

Platelet rich plasma (PRP) preparation were processed within 1 hour to the COVID19 and healthy samples. PRP was prepared by centrifuged 5 ml whole blood at 150 × g for 20 min at 20°C in the present of prostaglandin E1 (1μM) and apyrase (0.1unit mL−1) to prevent activation for next centrifugation. Platelets rich plasma were concentrated by centrifugation at 500×g for 15min. Platelets were leukocyte reduced and isolated by using human CD45+ depletion kit.

The Cyclooxygenase activity was evaluated in the platelets from PRP of all groups as mention below using ELISA technique.

3. Group design

1. Control group: 10 samples of healthy platelets suspension.
2. COVID-19 group: 20 samples of COVID-19 platelets suspension samples.
3. SIRT1 aptamer group: 20 samples of COVID-19 platelets suspension samples after treatment with 1μM SIRT1 aptamer.
4. Resveratrol group: 20 samples of COVID-19 platelets suspension samples after treatment with 40μM Resveratrol.
5. COX-1 and COX-2 inhibitors groups: 20 samples of COVID-19 platelets suspension samples after treated with 0.02 nM SC560 (COX-1 inhibitor) and 0.02 nM Celecoxib (COX-2 inhibitor) as a positive control.

The ability of Linear BAS SIRT1 aptamer as compared to Resveratrol (SIRT1 activator control) to inhibit COX-1 and COX-2 isoenzymes were determined using a cyclooxygenase activity assay (Fluorometric) kit. This is is very sensitive technique to detect the COX activity in biological samples. Test performed as the following:

A. Prepared standard curve dilution (0-20 pmol/well) by used 1 μM (1 pmol/μL) Resorufin standard.

B. 18 μL from 1×10^4 COVID19 platelet cells were added to 80 well in 96 well plate (5 μL platelets suspension +13 μL COX assay buffer). 2μL from 1 μM of SIRT1 aptamer and 40 μM Resveratrol were added to the plates as samples. According to the inhibitor controls, 2 μL from 0.02 nM SC560 (COX-1 inhibitor as a positive control) and 2 μL from 0.02 nM Celecoxib (COX-2 inhibitor as a positive control) were added to the inhibitor wells for the COX enzyme, and 2 μL of vehicle (DMSO, as total COX activity) was added to 100% initial activity and background wells.

C. The plate was incubated for 30 min at 37 °C . Starting the reaction by adding 68 μL of reaction mixture (2μl COX Probe + 4μl COX Cofactor + 52μl COX assay buffer) into each well, then immediately inject 10 μL of diluted cold arachidonic acid/NaOH solution to all the reaction well. Fluorescence measurement just after adding arachidonic acid at (Ex/Em = 535/587 nm) in a kinetic mode every 15 second one time. at 30 minutes because two time points (T1 and T2) in the linear range needs to calculated the COX activity of the sample (RFUS) and sample with inhibitor (RFUI).

Activity of COX is calculated as

ΔRFU535/587nm = (RFUS2 – RFUS1) – (RFUI2 – RFUI1)

Then, activity of COX in the test samples is calculated as:

$$COX\ Activity = (B/(\Delta T \times M)) = pmol\ min/mg\ or\ \mu U/mg$$

*Equations details are available in the kit leaflets.

4. Statistical analysis

All data statistical analysis was performed using Prism 8 software. Expression of results as means ± SEM. Analysis of the responses of concentration-dependent was by one-way analysis of variance (ANOVA) followed by Bonferroni's correction for multiple groups comparison. P-values of <0.05 were considered significant. The following Data represents three independent experiments.

5. Results

The inhibitory effect of SIRT1 aptamer on

Cyclooxygenase activity: One of the mechanisms of suppressing platelet aggregation was studied by virtue of Linear BAS SIRT1 aptamer ability to inhibit Cyclooxygenase activity. Healthy samples levels were measured as control group, COVID-19 group and different COX inhibitors groups (COVID-19 samples) were measured for comparision. The results as shown in figure 1 showed the Linear BAS SIRT1 aptamer significantly inhibits COX activity as similar as the inhibitory activity of SC560 (COX-1 inhibitor)($p< 0.001$). Resveratrol on other hand showed less decrease in the COX activity.

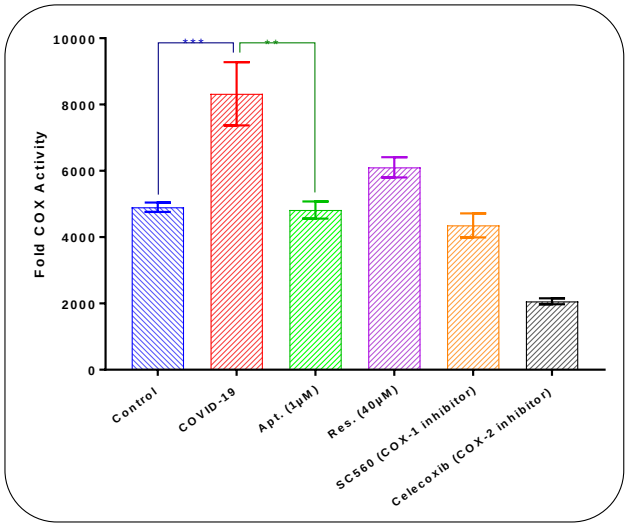

Figure 1: Measurement the inhibitory effect of COX activity by SIRT1 aptamer and resveratrol with COX activity assay Kit. COX activity was decreased with 1 μM of SIRT1 aptamer as similar as the inhibitory activity of SC560 and Celecoxib (COX-1 and COX-2 inhibitors respectively as a positive control) at the same concentration.

6. Discussion

COVID-19 associated coagulopathy (CAC) is among the most critical conditions that threatens patients life, many anticoagulant drugs are included in guidelines to prevent CAC but most of them has many adverse effects and can't completely prevent the risk of thrombosis. In this study we measured COX levels in healthy and COVID-19 patients. COX inhibitors and SIRT1 activators were used in COVID-19 patients samples. As shown in figure 1 Linear BAS SIRT1 aptamer significantly inhibited Cyclooxygenase activity as similar as the inhibitory activity of SC560 (COX-1 inhibitor), both of them decreased the COX levels almost to the normal levels (healthy group levels). SIRT1 Linear BAS Aptamer effect in decreasing COX activity supports the studies that suggesting antithrombotic effect from SIRT1 activation. COX is involved in thrombotic events through TBX2 pathway , COX-1 inhibition helps in preventing thrombosis (20). These results confirms the important pharmacological effect of SIRT1 Linear BAS Aptamer in modulating the thrombotic cascade in COVID-19 patients. From the above data it can be concluded that the Linear BAS SIRT1 aptamer antithrombotic action may reduce the COVID-19 associated coagulopathy through interacting with COX-TX2 pathway(21). COX-2 decreased activity suggesting a promising anti-inflammatory role for SIRT1 activation in COVID-19 (13).

7. Conclusion

In conclusion SIRT1 activation by Resveratrol and more potently by linear BAS SIRT1 aptamer shows a significant lowering in COX activity in COVID19 patient samples which predicts a promising protective antithrombotic effect against COVID19 associated coagulopathy. Linear BAS SIRT1 aptamer is less toxic and more potent than resveratrol to be considered as potential therapy (to be investigated) in CAC protection in COVID-19 patients.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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