Aspirin use Reduces Platelet Hyperreactivity and Degranulation in COVID-19 Patients

Younes Zaid, PhD1,2,3,* Qamar Lahlimi, PhD1,* Loubna Khalki, PhD4 Nabil Zaid, PhD1 Mounia Oudghiri, PhD3 Amine Cheikh, PhD, PharmD2 Abdallah Naya, PhD3 Yahye Merhi, PhD4 Fadila Guessous, PhD5

1 Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, Rabat, Morocco
2 Research Center of Abulcasis University of Health Sciences, Rabat, Morocco
3 Immunology and Biodiversity Laboratory, Department of Biology, Faculty of Sciences, Hassan II University, Casablanca, Morocco
4 Laboratory of Thrombosis and Hemostasis, Research Centre, Montreal Heart Institute, Montreal, Quebec, Canada
5 Faculty of Medicine, Mohammed VI University of Health Sciences (UM6SS), Casablanca, Morocco

Address for correspondence Younes Zaid, PhD, Research Center of Abulcasis University of Health Sciences, Allal Al Fassi Boulevard, Rabat 10000, Morocco (e-mail: y.zaid@um5r.ac.ma).

Semin Thromb Hemost

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is spreading throughout the world at an alarming rate.1 Severe disease is characterized by acute respiratory distress syndrome (ARDS), frequently associated with thrombotic complications.2,3 Some studies report the presence of platelets in thrombi found in multiple organs of COVID-19 autopsy cases.4,5 Moreover, hyperreactive platelets and overwhelming generation of inflammatory cytokines (i.e., a condition also known as “cytokine storm”) are frequent in COVID-19 patients.6–11 Furthermore, SARS-CoV-2 ribonucleic acid (RNA) could be detected in platelets of COVID-19 patients.6,10,12 Despite these findings suggesting a role for platelets in the pathophysiology of COVID-19, the mechanisms of increased thrombotic events remain not completely elucidated.

Aspirin is a typical nonsteroidal anti-inflammatory drug. Among its actions, the leading is the irreversible inhibition of cyclooxygenase-1 enzyme implicated in biosynthesis of the lipid mediator thromboxane A2 (TxA2). As TxA2 produced by platelet cyclooxygenase-1 is extremely potent at inducing platelet aggregation, aspirin is used as an antiplatelet agent in individuals at risk of cardiovascular diseases. Long-term low-dose aspirin (75–150 mg daily) can effectively reduce the incidence of recurrent ischemic cardiovascular and cerebrovascular events.13 However, prior studies demonstrate that long-term treatment (2 to 24 months, 100 or 330 mg/day) may be associated with progressive reduction in platelet sensitivity to this drug.14 Moreover, while aspirin can effectively reduce platelet activation induced by G-protein-coupled receptor signaling, it does not inhibit all platelet functions. Hence, studies have shown that the platelet role in maintenance of vascular integrity in inflammatory conditions involves immunoreceptor tyrosine-based activation motif (ITAM) but not TxA2.15,16

TxA2 is one of the most abundantly expressed lipid mediators found in the lungs of COVID-19 patients.17 Given the widespread use of aspirin, studies were performed to determine its potential prophylactic actions in COVID-19.18,19 In COVID-19, aspirin was shown to significantly diminish intensive care unit (ICU) admissions and need for mechanical ventilation.18 Its use was also associated with lower disease duration and mortality,19 which is in accord with a possible clinical benefit of early aspirin administration for prevention of ARDS.20 Whether aspirin given during infection can reduce platelet activation in COVID-19 and whether is safe and beneficial for patients with COVID-19 requires further investigation. During COVID-19 episodes, taking aspirin offers an affordable and easily accessible way to limit the lipid storm of TxA2 and its putative burden. Therefore, in this pilot investigation, we verified the effects of aspirin on platelet activation and aggregation in COVID-19 patients.

Participants were considered healthy if they did not require long-term medical therapy, had refrained from drugs...
known to influence platelet function during the previous 2 weeks, and did not have a history of bleeding symptoms. The female/male ratio of healthy donors was 3:7 and the mean age was 43.5 (58.50–26.50) years.

The inclusion criteria for COVID-19 patients were age > 18 years and voluntarily intake of aspirin (or not) as main treatment during SARS-CoV-2 infection. The admission period to Cheikh Zaid Hospital (CZH) was from December 7, 2020 to March 8, 2021.

The patient cohort was systematically assigned into two groups, that is, low-dose (100–150 mg daily) aspirin-treated group (Aspirin, $n = 10$) and nonaspirin-treated group (No Aspirin, $n = 10$). All patients were hospitalized and started treatment (aspirin or not) at admission on day 0 in the ICU.

Recruitment was approved by the Ethics Committee of CZH (CEFCZ/PR/2020/PR04) in compliance with the Declaration of Helsinki. All participants provided written informed consent.

Fresh venous blood was collected from healthy volunteers and COVID-19 patients on day 0 and at the indicated time points in accordance with guidelines of the Ethics Committee of CZH of Rabat. Washed platelets were prepared as previously described.\(^{\text{21,22}}\)

Aggregation of washed platelets was monitored on an eight-channel optical aggregometer (SD Medical Innovation, France) under stirring conditions (1,000 revolutions per minute) at 37°C in the presence of α-thrombin (Sigma Aldrich, USA) or collagen (Chronolog, USA). Platelet aggregation was recorded until trace stabilization and light transmission was measured at time of maximum aggregation.

Results are presented as mean ± standard deviation of 10 independent experiments. Statistical comparisons were performed using a one-way analysis of variance, followed by a Dunnett’s $t$-test for comparison against a single group. A $p$-value of $< 0.05$ was considered significant.

Platelet hyperreactivity, as determined by increased platelet aggregation in response to suboptimal concentrations of thrombin or collagen, is reported in COVID-19 patients at the time of hospital admission.\(^{\text{6,9,23}}\) Evaluation of aggregation induced by 0.05 U/mL thrombin and 0.5 μg/mL collagen revealed that platelets isolated on day 0 exhibited enhanced aggregation in COVID-19 patients versus healthy individuals ($n = 10$) ($\text{Fig. 1A, D}$), as previously reported.\(^{\text{6,9,10,23}}\) We then compared ex vivo platelet aggregation measured in healthy individuals with that of platelets from COVID-19 patients throughout the duration of the trial. We observed that hyperreactivity was consistent in nonaspirin-treated patients on day 7 and 15 in response to suboptimal dose of thrombin, and on day 7, 15, and 30 in response to low doses of collagen ($\text{Fig. 1A, D}$). This suggests that hyperreactivity is maintained in these individuals for extended duration and likely throughout the course of disease.

Conversely, aspirin failed to inhibit platelet aggregation initiated by higher concentrations of thrombin and collagen ($\text{Fig. 1B, E}$). Consumption of aspirin showed an early reduction in TxB\(_2\) (the stable metabolite of TxA\(_2\)) release beginning as early as day 7 that persists on day 15 and 30, in response to low-dose thrombin (0.05 U/mL) ($\text{Fig. 1C}$). Following activation with low-dose collagen (0.5 μg/mL),

**Fig. 1** Aspirin treatment reduces platelet hyperreactivity to thrombin and collagen. Platelets were isolated from healthy donors ($n = 10$, green dots), COVID-19 patients treated with aspirin ($n = 10$, red dots), or not treated with aspirin ($n = 10$, blue squares). Quantification of healthy controls is shown as green dots (A and D) or as salmon overlay (B and E). (A–C) Platelets were stimulated for 5 minutes at room temperature with 0.05 or 0.2 U/mL of α-thrombin. (A and B) Quantification (%) of maximal platelet aggregation and (C) quantification of inactive/stable thromboxane A\(_2\) metabolite TxB\(_2\). (D–F) Platelets were stimulated for 5 minutes at room temperature with 0.5 or 5 μg/mL of collagen. (D and E) Quantification (%) of maximal platelet aggregation and (F) quantification of inactive/stable thromboxane A\(_2\) metabolite TxB\(_2\). Data are represented as mean ± standard deviation (SD). Statistical analysis: One-way analysis of variance (ANOVA) with subsequent Dunnett’s $t$-test for comparison against a single group. ‘$p$ < 0.05’, ‘$p$ < 0.01’, ‘$p$ < 0.001.’
the production of TxB₂ was significantly reduced in aspirin-treated platelets only on day 7 and 15, but this difference was lost by day 30 (►Fig. 1F).

Thus, an aspirin regimen can reduce platelet aggregation induced by low concentrations of thrombin and collagen and the production of TxB₂, but the effects of aspirin on platelet aggregation are lost with higher concentrations of agonists.

We then investigated in vivo evidence of platelet activation in these patients. Higher levels of platelet factor 4 (PF4) and serotonin are reported in COVID-19, reflecting degranulation of α and dense granule components, respectively.7,24 Thus, we determined the plasma and platelet levels of PF4 and serotonin in these patients. As expected, elevated levels of PF4 and serotonin were observed on day 0 and 7, with levels progressively decreasing by day 15 (►Fig. 2A, B) to concentrations expected in healthy individuals.7 Aspirin efficiently reduced the concentrations of both circulating PF4 and serotonin by day 7 (►Fig. 2A, B). No inhibition was observed on day 15 and 30 (►Fig. 2A, B), which is likely explained by the absence of degranulation at these time points. Of note, the significantly higher content of PF4 and serotonin in aspirin-treated platelets (►Fig. 2C, D) is consistent with reduced degranulation of platelets in COVID-19 patients treated with aspirin.

Potential efficacy of aspirin in COVID-19 was reported.25,26 Moreover, accumulating evidence points to an association between aspirin use and decreased rates of mechanical ventilation, ICU admission, and in-hospital mortality.18,19 Nevertheless, mechanistic studies of the role of aspirin in the pathophysiology of COVID-19 have yet to be conducted. Given that aspirin was found without effect on platelet functions in certain proinflammatory conditions,15,16,27 and since it is unclear what mediates platelet activation in COVID-19, the question of whether aspirin would have any effect on platelet activation in COVID-19 patients remained to be addressed.

In addition to its well-established role of reducing inflammation28 and platelet function,29 antiviral effects against both deoxyribonucleic acid and RNA viruses have also been proposed for aspirin.30 In this pilot study, we found that aspirin can inhibit platelet aggregation induced by suboptimal concentrations of agonists and can reduce degranulation in vivo.

We confirmed that platelets isolated from COVID-19 patients are hypersensitive to suboptimal concentrations of agonists.
of thrombin, consistent with other studies. Platelets may be hypersensitized to agonist stimulation due to (1) higher expression of activation receptors, (2) activation of pathogen-recognition receptors or cytokines, (3) changes in platelet signaling regulation by ITAM (immunoreceptor tyrosine-based inhibitory motif) receptors, or (4) a procoagulant state in plasma supporting thrombin-activity itself. Although aspirin treatment interfered in platelet activation as shown by studies, the underlying mechanism of platelet activation in COVID-19 remains to be established.

In conclusion, our data suggest that aspirin may have an effect to attenuate platelet activation and aggregation in COVID-19. Given the relative safety of aspirin, this study warrants further investigation into possible beneficial effects of this drug in COVID-19.

Notes
The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author. Written informed consent was obtained from the individual(s) for the publication of any data included in this article.

Authors’ Contributions
Y.Z., Q.L., A.N., and F.G. designed the research. Y.Z., L.K., N.Z., and M.O. performed the research and statistical analyses. Y.Z., A.C., Y.M., and F.G. analyzed the data. All authors contributed to writing the paper.

Funding
The study was supported by the Cheikh Zaid Foundation-Rabat (awarded PRO42020 to Y.Z.).

Conflict of Interest
None declared.

References
27. Cloutier N, Allaey As, Marcoux G, et al. Platelets release pathogenic serotonin and return to circulation after immune complex-
28 Arif H, Aggarwal S. Salicylic Acid (Aspirin). Treasure Island, FL: StatPearls; 2021
30 Glatthaar-Saalmüller B, Mair KH, Saalmüller A. Antiviral activity of aspirin against RNA viruses of the respiratory tract—an in vitro study. Influenza Other Respir Viruses 2017;11(01):85–92