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The Effect of Aspirin on the World's Pandemic COVID-19

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Abstract

Aspirin has become one of the most frequently used and cheapest drugs in medicine now. Since its first synthesis in 1897, several medicinal roles and mechanisms of action of Aspirin have become apparent. Since its emergence, the COVID-19 pandemic has been ravaging the medical and economic sectors even with significant vaccination advances. In severe presentations, the disease of SARS-CoV-2 can manifest in life-threatening thromboembolic and multi-organ repercussions provoking notable morbidity and mortality. Aspirin, due to its well-known properties and multiple molecular targets, and ought to its extensive clinical use, has been perceived as a potential therapeutic agent for COVID-19. Aspirin acts at multiple cellular targets to achieve its anti-inflammatory and anti-platelet effects. Although initial promising clinical data describing aspirin's role in COVID-19 has appeared, evidence supporting its use remains fragile and premature. In this article, we highlight the history of Aspirin, a novel mechanism of action, and its uses. Also included is a brief statement of emerging new applications and principal mechanisms by which Aspirin inhibits acute inflammation and alters platelet biology; therefore, hypothesized that Aspirin might prove highly beneficial as a novel therapeutic drug for combating severe acute inflammation and thrombosis associated with the cytokine storm in COVID -19 patients.

Key words: Keywords: Aspirin, Mechanism of Action, Effect, COVID-19

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1. Introduction

Aspirin as a non-steroidal anti-inflammatory drug that has potent antiplatelet actions was first produced commercially in 1899 and next year marks its 100th anniversary. Aspirin is regarded as one of the most important drugs of the century, yet its use dates back to the ancient Egyptians, who knew about the pain-relieving properties of plants (Vane et al., 1992). Hippocrates (460-377 BC) knew of the pain-relieving effect of the juice obtained from the bark of the willow tree and in the Middle Ages, the bitter extract from boiled willow bark was a popular remedy for pain (Vane et al., 1992). In 1838, salicylic acid was isolated from the glycoside salicin and was identified as the active ingredient of willow bark (Chapman and Hall., 1996). Acetylsalicylic acid was first synthesized in 1853 by Von Gerhardt, by mixing salicylic acid with acetic acid. In 1894, Felix Hoffmann, a pharmacist at the Bayer company in Elberfeld, Germany, gave acetylsalicylic acid to his father to treat rheumatoid arthritis; formal clinical trials soon followed (Dreser et al., 1899). In 1899, acetylsalicylic acid was patented by Bayer Co. under the trade name of Aspirin: 'a' stood for acetyl and 'spir' stood for Spirsaure (a German word for salicylic acid). So overwhelming was the popularity of acetylsalicylic acid, which its original trade name eventually became the generic name. Initially, aspirin was used as an analgesic and an antipyretic. It was thought that aspirin affected the thalamus, thereby increasing the pain threshold. The anti-inflammatory properties of aspirin were subsequently recognized in 1971. The mechanism of action of aspirin—the inhibition of prostaglandin synthesis— was elucidated by Sir John Vane (Vane et al., 1971), who was awarded the Nobel Prize in 1982 for this important contribution to medicine.

Apart from analgesic, antipyretic and anti-inflammatory properties, several other therapeutic roles of Aspirin have become apparent since then. These include its use as an antithrombotic agent in the prevention of cardiovascular diseases (CVD) due to antiplatelet effects (Thorat et al., 2013; Vane et al., 2003) and, more recently, as an agent for cancer prevention and treatment. Cancer and CVD comprise a substantial proportion of the global disease burden and are the leading causes of disability and death in the developed world (Cuzick et al., 2013; Cuzick et al., 2009). Aspirin has considerable potential to reduce these if used prophylactically in the general population. However, Aspirin is also associated with excess bleeding, particularly gastrointestinal (G.I.) bleeding and hemorrhagic stroke being the most significant concerns. Evaluation of Aspirin as a prophylactic measure for the general population requires a careful assessment of both benefits and harms. It is essential to recognize that although relatively common, the vast majority of Aspirin related adverse effects (excluding intracranial bleeding) do not have long-term sequelae and are rarely fatal, especially for individuals under the age of 70 years (Cuzick et al., 2013; Patrono et al., 2005). The rapid development of coronavirus disease-2019 (COVID-19) as an acute lung injury/acute respiratory distress syndrome (ALI/ARDS) progressing to death has become a daunting challenge to manage. Options such as treatment with antiviral, anti-inflammatory, and anticoagulant agents and mechanical ventilation may not be readily available in underserved communities and remote areas of the world.

Within this framework, the degree of efficacy of most strategies assessed individually is variable ranging from low to moderate (Bikdeli et al., 2020; Cuker et al., 2021). Aspirin can influence different disease-relevant pathways during COVID-19. The interplay of these distinct aspirin-mediated effects may contribute to the outcome improvement of COVID-19 patients by interfering with the viral replication as well as its anti inflammatory and antithrombotic properties (Bianconi et al., 2020). Currently available antiviral agents and other novel therapies that directly target the virus may exhibit limited effectiveness over time due to adaptive mutations of the viral genome (Grabowski et al., 2021).

A limitation of virus-directed therapies may have a substantial impact on global health due to continued reports of mutant variants affecting repeated waves of COVID-19 around the world, which may further undermine the efficacy of these therapies that are also expensive (Grabowski et al., 2021). Therefore, it is impor-

tant to explore economically feasible and readily accessible resilient mechanisms to target COVID-19. Such strategies will facilitate the treatment of patients with COVID-19 and viral mutants in remote parts of the world and underserved communities. The scope of this article is to provide a comprehensive description of the rationale and supporting clinical evidence of aspirin as a multimodal therapeutic option for COVID-19.

2. Aspirin Pharmacology

Salicylic acid (Aspirin) is produced and administered via different routes in various doses and forms (Arif and Aggarwal., 2021). The usual therapeutic range of serum salicylate concentration is 10-30 mg/dl (0.7–2.2 mmol/L). Indeed, the dosing of aspirin is crucial as it dictates its mechanism of action. Traditionally, antithrombotic effects are achieved at low doses (75–81 mg/day), analgesic and antipyretic effects are achieved at intermediate doses (650 mg–4 g/day), while aspirin at high doses (between 4 and 8 g/day) is effective as an anti-inflammatory agent (Pillinger et al., 1998). Aspirin intoxication can occur after ingesting 10–30 g in adults and as little as 3 g in children. Most patients exhibit signs and symptoms of intoxication if the serum concentration level of salicylate exceeds 40–50 mg/dl (2.9–3.6 mmol/L) (Hill et al., 1973).

2.1 Pharmacokinetic

Acetylsalicylic acid is in general rapidly and completely absorbed by the gastrointestinal tract following oral administration (Awtry and Loscalzo., 2000). However, absorption may also be variable depending on several factors including the route of administration, the dosage, the rate of tablet dissolution, gastric pH, gastric contents, and emptying time (INC et al., 2017). It is mainly absorbed in the stomach and small intestine. The plasma concentration of salicylate peaks between 1 and 2 h following administration. It gets distributed to all body tissues shortly after administration, mainly to peritoneal, spinal, and synovial fluids, milk, saliva, liver, kidneys, heart, and lungs. It is also known to cross the placenta (DrugBankonline et al., 2005). Aspirin is hydrolyzed in plasma to salicylic acid and its levels become undetectable 4–8 h after administration. The liver is the main site of metabolism for salicylate, although other tissues may also be involved. It then gets eliminated by the kidneys via glomerular filtration and tubular excretion processes (INC et al., 2017). An entire dose needs around 48 h for the salicylate to be eliminated. The half-lives of ASA versus salicylate is 15 min versus 4 h respectively, while the clearance rate of ASA is variable depending on several factors (DrugBankonline et al., 2005).

2.2 Pharmacodynamic

Aspirin is unique due to its pharmacodynamics aspect, as it doesn't interact with any surface or intracellular receptors. It exerts its activity through non-specific irreversible acetylation of molecules. The acetylation process instigates alterations at the level of macromolecules and accordingly adjusts the function of the proteins. Due to the irreversibility of such modification, the duration of activity depends on the turnover rate of the target molecule irrespective of aspirin plasma concentration (Schrör et al., 2016).

3. Novel mechanism of action of aspirin

Aspirin is one of the most commonly used drugs worldwide (Vane and Botting et al., 2003; Zhou et al., 2014). It is an anti-inflammatory, antipyretic, analgesic, and anti-platelet drug. Aspirin exerts its major activity by inhibiting the cyclooxygenase enzyme (COX), which exhibits two forms: COX-1 and COX-2 (Patrono et al., 2001). Subsequently, it blocks the conversion of arachidonic acid into prostaglandins and thromboxane, collectively called prostanoids. Its activity expands to target several other structures circumventing a set of inflammatory and thrombotic events. The antithrombotic action of aspirin depends on the irreversible inhibition of arachidonate cyclo-oxygenase activity in platelets, thereby reducing the extent of thromboxane A2 (TXA2) formation and consequently the aggregability of platelets. The formation of intra-arterial thrombi is thus reduced. Aspirin affects a balance between TXA2, which is released from platelets, and prostacyclin, which is made by the blood vessel walls.

3.1 Cyclooxygenase (COX) pathways

It is well documented that "aspirin irreversibly inhibits cyclooxygenase (COX) by acetylation of

an amino acid serine residue, as seen in Figure 1. Also, it blocks the subsequent biosynthesis of prostaglandins and thromboxane" (Alegbeleye et al., 2020; Chiang et al., 2009). "COX has at least two forms, COX-1 and COX-2. COX1 is the main form present in mature platelets in the blood, where it transforms arachidonic acid to the intermediates PG-G/H, which are subsequently converted to thromboxane A2. Thromboxane A2 is a vasoconstrictor and potent platelet activator. Thus, inhibition of thromboxane A2 formation explains Aspirin's anti-thrombotic properties," (Alegbeleye et al., 2020; Chiang et al., 2009). In the early 1990s, the second form of COX was identified, namely, COX-2. COX-2 was initially conceptualized as an "inducible" COX that is elevated in its quantity by a wide range of agents that stimulate inflammation or cell division and seems to be responsible for local formation during inflammation and cancer. Many recent studies demonstrated that low-dose aspirin evokes beneficial effects not only in the prevention and treatment of cardiovascular diseases, but also in decreasing the incidence of lung, colon, and breast cancers, and perhaps Alzheimer's disease (Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004). Although inhibition of prostaglandins and thromboxanes can account for aspirin's therapeutic benefits, aspirin's ability to regulate neutrophil-mediated inflammation remains of interest (Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004). Hence, low-dose aspirin effects that go beyond the inhibition of prostaglandins and thromboxanes are becoming increasingly apparent. In this regard, Aspirin, to its well-appreciated ability to inhibit prostaglandins and thromboxanes, can also 'switch on the production of the body's anti-inflammatory lipid mediators, namely, aspirin-triggered lipoxins (ATL) (Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004). "This novel class of mediators functions as local 'braking signals' in inflammation and actively participates in dampening host immune responses and quickly bringing the inflammatory reaction to a closure, a process called resolution. Thus, they may account at least in part for Aspirin's clinical benefits distinct from Aspirin's anti-thrombotic action,"(Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004).

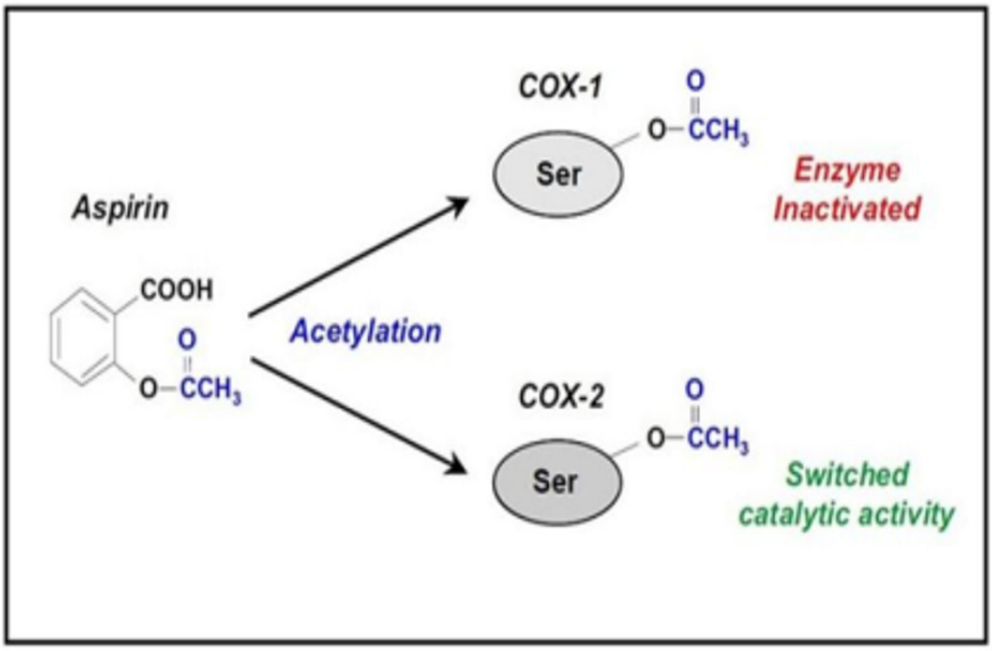

Fig 1. The novel mechanism of action of Aspirin. COX: cyclooxygenase; SER: serine

Many recent studies demonstrated that low-dose aspirin evokes beneficial effects not only in the prevention and treatment of cardiovascular diseases, but also in decreasing the incidence of lung, colon, and breast cancers, and perhaps Alzheimer's disease (Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004). Although inhibition of prostaglandins and thromboxanes can account for aspirin's therapeutic benefits, aspirin's ability to regulate neutrophil-mediated inflammation remains of interest (Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004). Hence, low-dose aspirin effects that go beyond the inhibition of prostaglandins and thromboxanes are becoming increasingly apparent. In this regard, Aspirin, to its well-appreciated ability to inhibit prostaglandins and thromboxanes, can also 'switch on the production of the body's anti-inflammatory lipid mediators, namely, aspirin-triggered lipoxins (ATL) (Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004). "This novel class of mediators functions as local 'braking signals' in inflammation and actively participates in dampening host immune responses and quickly bringing the inflammatory reaction to a closure, a process called resolution. Thus, they may account at least in part for Aspirin's clinical benefits distinct from Aspirin's anti-thrombotic action,"(Alegbeleye et al., 2020; Chiang et al., 2009; Serhan et al., 2004).

3.2 Mechanism of Platelets Action in Hemostasis

3.2.1 Platelet Activation and Thrombogenesis

Upon injury of the blood vessel intima, as it occurs after trauma or rupture of an atherosclerotic plaque, subendothelial collagen and von Willebrand factor (vWF) are exposed to circulating blood components. Platelets adhere to both collagen and vWF on the injured endothelium through their glycoprotein Ia/IIa and Ib/V/IX receptors, respectively, eliciting the release of calcium (Kasotakis et al., 2009; Furie et al., 2008). Calcium induces a conformational change in the platelet glycoprotein IIb/IIIa (gp IIb/IIIa) receptors, so they can bind circulating fibrinogen molecules. Calcium also stimulates the release of alpha-granules and dense granules. P-Selectin, one of the proteins released from alpha-granules, mediates monocytes' adhesion and neutrophils to activated platelets (Palabrica et al., 1992). This function is integral to the recruitment of leukocytes into newly-formed thrombi, the perpetuation of thrombogenesis, and the overall hemostatic process. Dense granules release adenosine diphosphate (ADP), which further perpetuates platelet activation by binding to ADP-specific receptors (P2Y1) and promotes the action of phospholipase A2 on membrane phospholipid compounds to produce arachidonic acid (ARA). Arachidonic acid is subsequently converted into thromboxane A2 (TXA2) and prostaglandins (mainly G2 and H2) within platelets, a conversion mediated by thromboxane synthase and the cyclooxygenase (COX) isoenzymes 1 and 2, respectively (Espinosa et al., 2012). TXA2 is the most critical platelet activator and functions by inducing expression of fibrinogen receptors (gp IIb/IIIa) on the platelet membrane and binding to TXA2 receptors on the surface of other platelets triggering their activation. It also plays a secondary, but equally important role in hemostasis, as a potent vasoconstrictor. Platelet activation also occurs with the attachment of other freely circulating nascent compounds, such as ADP, fibrinogen, thrombin, adrenalin, and prostaglandin I2, to corresponding ligand-specific receptors (Roth et al., 1994).

3.2.2 Aspirin effect on Platelet Activation

Aspirin irreversibly inhibits COX-1 in platelets by acetylating its serine-529 residue, thereby blocking TXA2 and other eicosanoid production from ARA. TXA2 is the most significant trigger for platelet activation. Because platelets lack a nucleus and therefore are deprived of protein synthetic ability, this inhibition cannot be overcome by new COX-1 synthesis and lasts for the platelet's lifespan (7-10 days). Aspirin-induced COX-1 inhibition is rapid, irreversible, and saturable at low doses (no dose dependent). After a single 325mg dose of Aspirin, platelet COX-1 activity is completely inhibited and recovers by about 10% per day, due to nascent platelet release in the circulation (Roth et al., 1994).

4. Mechanisms of virus entry into the host cells and inflammatory cascade

During the first steps of SARS-CoV-2 (severe acute respiratory syndrome- coronavirus-2) infection, spike (S-glycoprotein on the surface of the SARS-CoV-2 binds to the angiotensin-converting enzyme receptor 2 (ACE2). Transmembrane protease serine (TMPRSS) 2 is involved in S protein priming that facilitates virus entry into the host cell (endocytosis). The ACE2 receptor and TMPRSS2 are highly expressed on the surface of alveolar cells in the lower respiratory tract (Harrison et al., 2020). After viral release into the cytoplasm of the host cell, the SARS-CoV-2 viral genome produces two polyproteins (PPs). These PPs help control the host cell machinery in a "hostile takeover" resulting in their rapid translation and replication in endosomes (Merad et al., 2020). Mature virions are later released by exocytosis and can bind to TLR4 on the host cell membrane to trigger the activation of the intracellular inhibitor of kappa B kinase (IKK) complexes. The viral ribonucleic acid (RNAs) bind to Toll-like (TLR) receptors (TLR3 for double-stranded RNAs or TLR7/8 for single-stranded RNAs) on endosomal membrane leading to the activation of transcription of the interferon-regulatory factor (IRF) family and subsequently type I interferon and also induce NF-κB (nuclear factor-κB) activation (Figure 2). The activation of IKK results in the phosphorylation of the cytoplasmic inhibitor factor, IkBα triggering its ubiquitination and degradation by the 26S proteasome. Simultaneously, NF-κB (a heterodimer complex consisting of protein subunits p50 and p65) is released from IkBα, then it translocates into the nucleus and induces transcription of various genes coding for pro-inflammatory proteins such as cytokines, chemokines, adhesion molecules, acute phase proteins, and growth factors (Kircheis et al., 2020). These cytokines activate inflammatory leukocytes (neutrophils, macrophages) and trigger their infiltration into the alveolar space further boosting the generation and release of large quantities of inflammatory cytokines in a "cytokine storm". Activated macrophages express tissue factor that activates coagulation leading to systemic hypercoagulability (Jayarangaiah et al., 2020). COVID-19-induced coagulopathy (CIC) is characterized by neutrophilia and elevated levels of interleukin-6, C-reactive protein, D-dimer, FVIII, and fibrinogen. The latter processes induce inflammatory cell activation and infiltration, vascular leakage, pulmonary edema, rapid progress to ARDS, multi-organ dysfunction, and death in a substantial percentage of patients. Similarly, cytokine storm and hypercoagulability are associated with pulmonary micro thrombosis and eventually apoptosis of pulmonary cells (Furlow et al., 2020).

5. Role of platelets in COVID-19

In addition, multiple lines of evidence point towards an essential role of platelets during COVID-19 ALI/ARDS. Platelets play a critical role in hemostasis, thrombosis, and innate immune and inflammatory responses. Platelet-leukocyte aggregate formation and abnormal leukocyte accumulation in the lungs, hypercoagulability, and elevated levels of thromboxane A2, P-selectin and biomarkers of inflammation have been demonstrated in animal models of ALI/ARDS (Rocca et al., 2020). Direct binding of SARS-CoV-2 to platelets through ACE2 and TMPRSS2 expressed on platelets has been demonstrated in in vitro studies. Elevated platelet aggregation, glycoprotein (GP) IIb/IIIa expression at the platelet surface, P-selectin expression, and platelet granule secretion has been demonstrated in ALI/ARDS models. Subsequently, activated platelets bind to leukocytes and enhance their infiltration into the lungs to further propagate inflammation (Zhang et al., 2020). Subjects suffering from viral upper respiratory tract infections have been shown to exhibit elevated platelet P-selectin expression and heightened platelet reactivity (Kreutz et al., 2007). It has been hypothesized that the thrombo-inflammatory state has an important influence on the outcomes of patients with cardiovascular disease and human immunodeficiency virus infection during and after percutaneous coronary intervention (Gurbel and Bliden., 2017). Megakaryocytes are precursors of platelets. Following their release from bone marrow, platelets circulate in large numbers through

the lungs, where they are dynamically released. A recent study using direct imaging of lung microcirculation in mice suggested that the lungs can contribute up to 50% of total platelet release. Since the lungs are severely affected during COVID-19, platelet generation/function may also be severely affected. In the presence of endothelial dysfunction, elevated levels of von Willebrand Factor (VWF) expression in the lung, elevated systemic levels of fibrinogen, and activated platelets likely play a significant role in pulmonary thromboembolic event occurrences. Activated platelets stimulate neutrophils to produce neutrophil extracellular traps (NETs). NETs consist of deoxyribose nucleic acid (DNA) fibers, histones, and antimicrobial proteins that help to entrap and degrade invading bacteria and viruses (NETosis) as part of the innate immune response. However, platelet-induced NETosis can be more lethal resulting in tissue damage, hypercoagulability, and thrombosis. In a prospective cohort study in patients with COVID-19, elevated levels of platelet–neutrophil aggregates, plasma myeloperoxidase (MPO)-DNA complexes, platelet factor 4, and Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTEs) have been demonstrated. Circulating NET levels correlated with the severity of COVID-19 (Janiuk et al., 2021). Furthermore, in autopsies, the presence of NET-containing microthrombi with platelet–neutrophil infiltration in lungs again highlights the critical role of platelets in COVID-19 (Janiuk et al., 2021). Post-mortem examination of patients with COVID-19 found a nine times higher prevalence of microvascular platelet-fibrin clots in the pulmonary vasculature than in patients with influenza virus infection.19 In vivo evaluation of the sublingual microcirculation in patients with severe COVID-19 requiring mechanical ventilation revealed microvascular thrombi in 85% of the cases. Finally, the presence of microthrombi in the heart, kidneys, and liver in patients with COVID-19 may indicate widespread thrombotic microangiopathy resulting in multiorgan dysfunction (Bikdeli, et al., 2020).

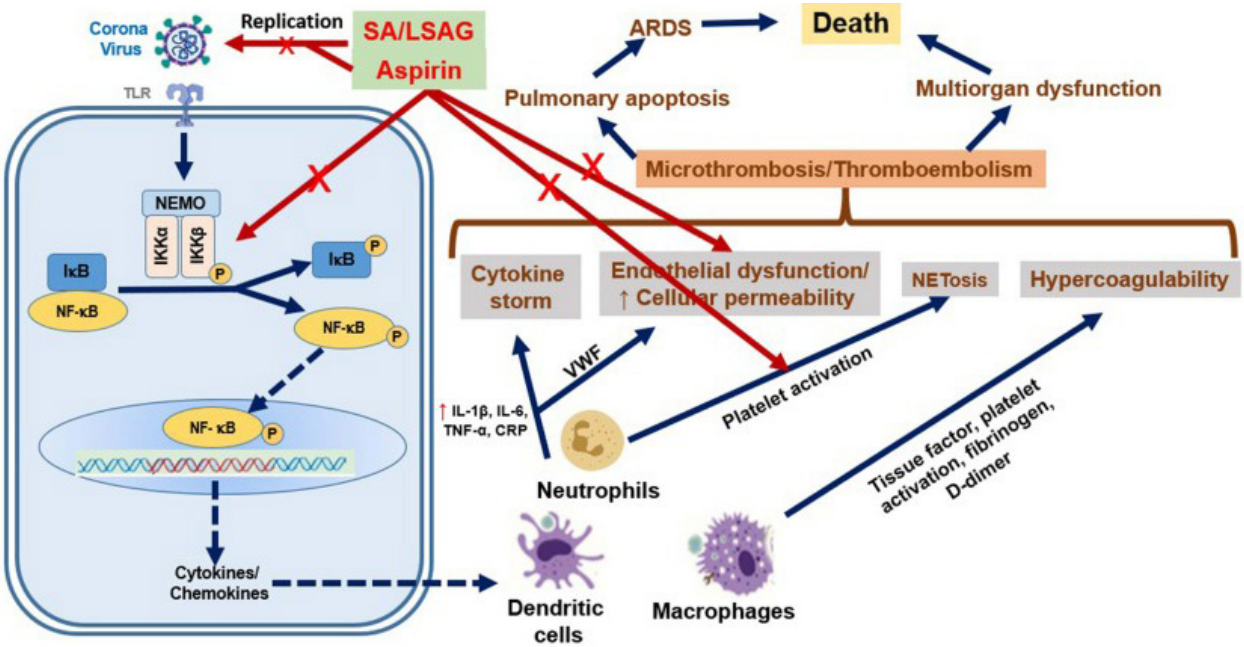

Figure 2. Host cell response to SARS-CoV-2 infection and role of aspirin in SARS-CoV-2 infection. SARS-CoV-2 induced cytokine storm, endothelial dysfunction, NETosis, and hypercoagulability result in microthrombosis/thromboembolism in lungs as well as heart and kidney leading to multiorgan dysfunction, ARDS, and ultimately death in a substantial percentage of patients. Aspirin/SA/LSAG can attenuate viral replication and inhibit NF-κB activation and subsequent expression of cytokines and chemokines. In addition, ASA exhibits anti-inflammatory and antiplatelet effects and attenuates NETosis, endothelial dysfunction, and hypercoagulability.
Abbreviations: ASA, acetylsalicylic acid; SA, salicylic acid; LASAG, D, L-lysine-acetylsalicylate glycine; NF-κB, nuclear factor-κB; IL, interleukin; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; MCP-1, macrophage chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; NETs, neutrophil extracellular traps; ARDS, acute respiratory distress syndrome.

6. Potential role of aspirin in COVID-19

The evidence totality consistently indicates that the resilience mechanism to target during COVID-19 for both prevention and treatment should involve effective and simultaneous attenuation of viral infection and replication, cytokine release, endothelial dysfunction, coagulation, fibrinolysis, and importantly, platelet function. Therefore, aspirin – an inexpensive, widely available, safe, and time-tested agent with anti-inflammatory, antithrombotic, and antiviral properties – can be a credible adjunctive therapeutic option for the treatment of COVID-19 (Gurbel et al., 2020). Most importantly, instead of targeting the virus directly, aspirin targets the intracellular signaling pathway of the host cell that is essential for viral replication, and resultant inflammatory responses, hypercoagulability, and platelet activation that follow infection. Thus, aspirin would be predicted to be effective even in the presence of mutant virus forms. The role of aspirin may be particularly relevant in situations where more expensive therapeutic options are not readily available (Arif et al., 2021).

Two major underpinnings for using aspirin in COVID-19 include the effect of aspirin on the inhibition of cyclooxygenase (COX)-1 and COX-2 enzymes by acetylation and the effect on NF-κB by salicylic acid (Tantry et al., 2009). The acetyl group of aspirin binds to the serine residue (Ser529) in platelet COX-1 irreversibly and non-competitively for the lifespan of platelets and thereby inhibits the eventual generation of an important platelet agonist, thromboxane (Tx) A2, and platelet activation. An antithrombotic effect of aspirin has been primarily attributed to the inhibition of the platelet COX-1 enzyme that occurs at low doses (75–81mg/day). In vitro studies indicate that the inhibitory potency of aspirin against COX-1 and COX-2 is similar in molar terms. However, in vivo higher molar concentrations of aspirin are required because of the significant protein (COX) turnover rate of nucleated cells as opposed to the missing turnover of the anucleated platelets. Thus, within the short half-life of aspirin in the circulation, amounting to 20–30 min, enough new enzyme protein might have been synthesized that escapes the acetylation by aspirin. COX-1 inhibition results in the direct inhibition of prostaglandin intermediate endoperoxide generation in the platelet that is upstream from the generation of TxA2 by thromboxane synthase (Azboy and Haddad et al., 2017). These inhibitory properties are reflected in reduced excretion of the stable thromboxane metabolite, urine 11-dehydro thromboxane B2 (u11-DH TxB2). Thus, u11-DH TxB2 represents the whole-body COX inhibitory response induced by aspirin. The independent relation of urine u11-DH TxB2 to adverse outcomes in patients with cardiovascular disease and diabetes treated with aspirin has been demonstrated in major clinical trials (Rocca et al., 2020). At higher doses, aspirin also inhibits the COX-2 enzyme that is expressed during inflammatory conditions by acetylating the homologous Ser516 residue. The latter effect results in the inhibition of pro-inflammatory prostaglandin E2 (PGE2) generation. The inhibition of COX-2 by aspirin also results in the inhibition of prostaglandin intermediate endoperoxide synthesis that participates in TxA2 generation through transcellular biosynthesis (Chen et al., 2020). Acetylated COX-2 can convert arachidonic acid to 15-epoxy-lipoxin A4, also known as aspirin-triggered lipoxin (ATL). Lipoxins and aspirin via ATL inhibit leukocyte-endothelial interactions by stimulating nitric oxide release, decreasing vascular permeability, and attenuating endothelial dysfunction by improving oxygen defense. In addition, the aspirin-acetylated COX-2 enzyme generates an aspirin-triggered resolving D1 (ATRvD1) molecule.39 In an acid-initiated murine lung injury model, administration of ATRvD1 significantly decreased bronchoalveolar lavage fluid neutrophils, platelet–neutrophil interactions, the release of cytokines and p-selectin, and nuclear translocation of NF-κB-phosphorylated p65 as determined by immunohistochemistry of lung sections. Aspirin and salicylic acid at 1–5 mM concentrations have been shown to inhibit NF-κB activation and NF-κB induced inflammatory cytokine generation. This involves transcriptional activation of differentially regulated transcription factors, including NFkB, and is apparently due to nonselective kinase inhibition. It has been demonstrated that aspirin and

salicylic acid inhibit IKK activity by blocking ATP binding to IKK-β. Aspirin has been shown to attenuate thrombin generation and factor XIII activation in a microvascular injury model as well as thrombin-induced venous thromboembolism via inhibition of thromboxane action (Azboy et al., 2017). Aspirin can also enhance fibrin clot permeability and clot lysis by acetylating lysine residues in fibrin at high doses. In addition, aspirin non-specifically acetylates a variety of proteins and nucleic acids at micromolar concentrations. In an in vitro experiment with phorbol 12-myristate 13- acetate (PMA)- or tumor necrosis factor-α (TNF-α)- activated human neutrophils, 5 mM aspirin was associated with a significant reduction in a NET release. This reduction was correlated with a significant reduction in the phosphorylation of the NF-κB p65 subunit indicating aspirin can attenuate NETs release from neutrophils by inhibiting NF-κB. Aspirin was shown to inhibit reactive oxygen species generation, neutrophil infiltration, macrophage generation, and lung edema in a hyperoxia-induced lung injury model in NF-κB -luciferase transgenic mice (Chen et al., 2020). Concerning the direct antiviral effects of aspirin, in vitro and in vivo studies have shown that aspirin can effectively block influenza virus infection. This antiviral effect has been attributed to the inhibition of viral replication and propagation through NF-κB inhibition. The antiviral effect of the D, L-lysine-acetylsalicylate glycine (LASAG), an aerosolized formulation of aspirin, is discussed later.

7. Aspirin benefit for thromboprophylaxis in COVID-19

As mentioned earlier, DIC and predisposition to thromboembolism are implicated in COVID-19. At the cellular level, activated platelet-neutrophil interaction is essential for the formation of neutrophil extracellular traps (NETs). Through a feed-forward cycle of NETs and platelet aggregation, NETs are proposed to induce adverse cardiac and kidney events in COVID-19 patients via the production of excessive thrombosis. Indeed, some studies advocate for thrombo-prophylaxis and the treatment of coagulopathy in COVID-19 patients (Belen-Apak et al., 2020). However, these studies mostly recommend low molecular weight Heparin for the management of COVID-19 patients with coagulopathy.

Interestingly, there is evidence that Aspirin could be used for thrombo-prophylaxis in post-operative patients of joint arthroplasty and prevent deep vein thrombosis in mechanically ventilated intensive care patients. Besides, Aspirin has been found to help in the prevention of thrombosis through the COX-dependent and COX-independent pathways. These pathways include acetylation of prothrombin and platelet membranes and impaired formation of NETs. Furthermore, compared to Heparin, Aspirin may have lesser side effects of anticoagulant-related bleeding. In light of the above, Aspirin becomes relevant as an adjuvant or monotherapy for the management of excessive thrombosis in COVID-19 patient care (Azboy et al., 2017).

8. Aspirin effect On COVID-19-associated inflammation

In most cases of COVID-19 disease, the stimulated immune system is capable of resolving the infection where it initially triggers a local immune response, followed by recruiting macrophages and monocytes that in turn release cytokines and prime adaptive T-cell and B-cell immune responses (Merad et al., 2020). Dysregulation of the inflammatory immune response which is associated with severe COVID-19 disease inhibits the development of protective immunity to the infection. They suggested that uncontrolled immune dysregulation, hypercytokinemia, ‘‘cytokine storm’ or macrophage activation syndrome is associated with ARDS, MOF, and mortality in certain populations (men, elderly, and individuals with comorbidities) (Manjili et al., 2020). Autoimmune conditions such as antiphospholipid syndrome (APS) and multisystem inflammatory syndrome in children (MIS-C) have been reported in patients with COVID-19. Cytokine storm in COVID-19 is associated with the elevation of pro-inflammatory cytokines and chemokines. These cytokines include interleukin (IL)-6, IL-2, IL-7, IL-8, IL-1β, interferon (IFN)-γ, tumor necrosis factor-α (TNF-α), granulocyte colony-stimulating factors, chemokines including

C-X-C motif chemokine ligand 10, C-X-C motif chemokine ligand 8 and chemokine (C-C motif) ligand 2 (Manjili et al., 2020). Because of hyper inflammation's role in COVID-19, therapeutic agents that target the inflammatory pathway have been employed. Aspirin is used in moderate and high doses in children with MIS-C to treat inflammation in the acute stage, and it has already been listed in 14 studies on the clinical trials website, including 10 randomized controlled trials. Other immunomodulatory therapeutics were also used including steroids, intravenous immunoglobulin (IVIG), anti-cytokine agents (IL-1 antagonist anakinra, IL-6 receptor antagonists tocilizumab, and sarilumab), antichemokine agents (e.g, cenicriviroc or leronlimab) and Janus kinase (JAK) inhibitors (eg, baricitinib or ruxolitinib). Despite a strong rationale and several previous promising open studies, a randomized controlled study to evaluate the safety and efficacy of tocilizumab in patients with severe COVID-19 pneumonia (COVACTA) failed to meet its primary endpoint of improved clinical status or to improve patients' mortality (Furlow et al., 2020). Another prospective randomized controlled trial about the use of sarilumab, registered as (CORIMUNO-VIRO), was suspended for futility (NCT04341870).

9. Aspirin Effect On COVID-19-associated endothelial dysfunction

Endothelial cells play an important role in the pathogenesis of ARDS and MOF in patients with COVID-19 (Varga et al., 2020). In other words, they contribute to the initiation and propagation of severe COVID-19 by inducing vascular endothelins, altering vessel barrier integrity and permeability, activating coagulation pathways, and deregulating inflammatory cell infiltration. Host-dependent cardiovascular (CV) factors or established cardiovascular disease (CVD) in addition to viral factors could contribute to the severity of COVID-19 disease in these patients who have chronic endothelial dysfunction (Teuwen et al., 2020). Varga et al found endothelial cell involvement across vascular beds of different organs in three patients with COVID-19 with CV comorbidity, who developed respiratory failure and MOF. The histological findings showed the presence of viral bodies within endothelial cells and a responsive accumulation of inflammatory cells, with evidence of endothelial and inflammatory cell death. COVID-19 endothelins in several organs are suspected to be the result of direct viral infection, host inflammatory response, host apoptosis, and pyroptosis (Varga et al., 2020). Pyroptosis and endothelial dysfunction were also demonstrated in the COVID-19 pulmonary samples, which may lead to systemic thrombosis. COVID-19-induced endothelins provide a reasonable sound for treatment modalities to stabilize the endothelium while tackling SARS-CoV-2 replication, especially with anti-inflammatory, anti-cytokine drugs, ACE inhibitors, and statins. Florêncio et al formulated a hypothesis that aspirin and statins play an essential role in preventing COVID-19- induced endothelins, and progression to severe forms (Florêncio et al., 2020).

Low-dose aspirin has a putative role in the secondary prevention of arterial thrombosis in patients with COVID-19 with established CVD, but its role in the primary prevention of atherosclerotic cardiovascular disease (ASCVD) with chronic endothelial dysfunction is controversial (Patrono et al., 2005). Aspirin has pleiotropic effects on endothelial function and assessing these effects during the COVID-19 era in further research will be very helpful for proper understanding and management of COVID-19 infection.

10. Aspirin Effect On COVID-19-associated coagulopathy

COVID-19-induced coagulopathy may, in part, be due to direct vascular damage induced by SARS-CoV-2 infection or ACE-2 inhibition; the latter is expressed on arterial and venous endothelial cells and plays an anti-inflammatory protective role. In severe and critical COVID-19, a bi-directional association of inflammation and coagulopathy may exist where persistent inflammatory status acts as an important trigger for the coagulation cascade and may promote an aggressive immune response. Certain cytokines, such as IL-6, could activate the coagulation system and suppress the fibrinolytic system. Inflammatory cytokines can contribute to a pro-coagulopathic state by several pathways, but the actual

mechanisms of inflammatory-induced coagulopathy in severe COVID-19 remain to be determined (Merad et al., 2020). Since the emergence of the COVID-19 pandemic, studies have shown an increased incidence of venous, arterial, and microvascular thrombosis in this population of patients, with a higher prevalence in the severe form of the disease. A literature review by Obi et al reported a 21%–69% incidence rate of venous thromboembolism (VTE) in critically ill patients with COVID-19 (Obi et al., 2021). In addition to deep venous thrombosis and acute pulmonary embolism (APE), patients developed circuit clotting through continuous renal replacement therapy and ECMO. They also described 'breakthrough VTE', where VTE develops despite receiving prophylactic or therapeutic doses of anticoagulation. In a cohort study of 198 hospitalized patients with COVID-19 in Amsterdam, where all patients had received prophylactic anticoagulation, the cumulative risk for developing VTE at 7, 14 and 21 days was 16%, 33%, and 42%, respectively, with a higher incidence in intensive care unit (ICU) patients than non-ICU patients. Previous studies found that VTE was the predominant macrovascular complication. A Dutch study reported the frequency of arterial thrombosis (ischaemic stroke, myocardial infarction (MI), or systemic thromboembolism) as 3.7% in 184 ICU patients. Whereas in a health system in New York, where most patients were treated with a low dose of anticoagulation, the incidence of arterial and venous thrombosis in 829 ICU patients were 18.6% and, 13/6 % respectively, and among 2505 non-ICU patients, the incidence of arterial and venous thrombosis were 8/4 % and 3.6% respectively. The presence of APE was not just restricted to severe or critical COVID-19, 18% of non-hospitalized patients with COVID-19 evaluated by CT pulmonary angiography in the emergency department were found to have APE (Gervaise et al., 2020). Multiple thromboembolic events have been also reported in outpatients with mild COVID-19 illness after developing extreme hypercoagulability status. The lack of universal guidelines for the early detection and treatment of COVID-19-associated coagulopathy in non-hospitalized patients might contribute to the scarcity of data about coagulation status in the outpatient setting. COVID-19 is also associated with antiphospholipid antibodies, which may manifest with macrovascular or microvascular thrombosis. Emergent evidence suggests that manifestations of severe COVID-19 mimic more the clinical phenotype and pathophysiology of complement-mediated thrombotic microangiopathies (TMA), rather than sepsis-induced coagulopathy or disseminated intravascular coagulation (DIC). COVID-19-associated coagulopathy presents initially with minimal abnormalities in PT and platelet count followed by increased D-dimer and fibrinogen. In DIC, D-dimer is minimally increased while the platelet count is markedly decreased than in patients with COVID-19. VTE and arterial thrombosis are more frequent in CAC compared with SIC/DIC. Clinical and laboratory features of CAC overlap somewhat with the haemophagocytic syndrome (HPS), APS, and TMA; however, patients with COVID-19 still might be at risk of developing DIC. To date, there is no evidence that aspirin was used to treat complement-mediated thrombotic microangiopathies. Nevertheless, early uncontrolled observations suggest good outcomes in patients with COVID-19 after the use of the C5 inhibitor eculizumab, the C3 inhibitor AMY-101, or anti-C5a antibody, which need further confirmation by randomized controlled trials (Merrill et al., 2020).

Postulated that early administration of low-dose aspirin in patients with COVID-19 represents a pivotal pharmacological strategy for the prevention of arterial thromboembolism and VTE by targeting the thrombo-inflammatory process (Diaze et al., 2020). Aspirin prevents and treats arterial thromboembolism and VTE through several mechanisms of action. The earliest events in thrombus formation are platelet adhesion followed by aggregation, platelet activation, and granule release.

Except for platelet adhesion, all of these platelet functions are inhibited by aspirin. Aspirin irreversibly inactivates cyclooxygenase-1 and suppresses the generation of prostaglandin H2 (a precursor of thromboxane A2), which results in inhibiting platelet aggregation. Other mechanisms of antithrombotic effects of aspirin include

suppression of platelet activation and release reaction, inhibition of neutrophil recruitment on vascular endothelium, reducing thrombin generation, inhibition of factor XIII activation, increasing plasma clot permeability, altering fibrin clot structure and increasing its density, in addition to enhancing fibrinolysis by acetalization of fibrinogen and fibrin (Mekaj et al., 2015).

11. Conclusion

Aspirin exerts plural effects that position it as an ideal pharmacologic agent to treat COVID-19. These effects include anti-inflammation, antithrombosis, and antiviral properties that are mediated through two key pathways: the inhibition of IKK kinase and the inhibition of COX. Other non-canonical pathways such as acetylation of endothelial nitric oxide synthase (eNOS) NOS, endothelial protection, and upregulation of heme oxygenase-1 that are involved in protection from oxygen radicals may also play a role in modulating the inflammatory and prothrombotic responses observed in COVID-19. The optimal dose of aspirin to treat COVID-19 is critically dependent on the disease state. In patients who are hospitalized for COVID-19, readily available high-dose aspirin (325 mg/day) in addition to other standard medications may attenuate high inflammation and thrombotic risk. Aspirin effects may be enhanced by aerosol delivery to the lung which is the key organ damaged in COVID-19. The inhaled formulation with rapid effects may be an effective strategy during the critical initial course of the disease, but studies are ongoing with these formulations. For long-term treatment in the post-COVID-19 scenario, 325 mg per day can be used to prevent recurrent thrombotic event occurrences. Further clinical studies are required to support this suggestion. However, a low dose (75–162mg/day) is not optimal to attenuate the high inflammation and platelet function as has been demonstrated in recent pharmacodynamic studies. Therefore, low-dose aspirin may not be associated with significant clinical benefits. The elevated bleeding risk associated with a higher dose of aspirin may be a major limitation. COVID-19 is a hypercoagulable disease with a lower risk for bleeding, the net outcomes may favor attenuating thrombotic risk. In the recent prospective recovery trial, aspirin use was associated with an elevated bleeding risk. Being a widely available, inexpensive drug with multiple proven benefits, aspirin can be exploited globally, particularly in underserved communities and remote areas of the world, to combat the ongoing COVID-19 pandemic. Furthermore, by being uninfluenced by viral mutagenesis in its unique mechanisms of action, aspirin holds the promise of being a strong ally in the fight against our new enemy and other viruses that will certainly follow.

The pandemic has imparted significant burdens on the global health and economic sectors, leaving behind substantial morbidity and mortality. While the scientific community merged efforts to obtain the vaccine at an un preceded velocity, the search for a therapeutic agent is still ongoing. Aspirin with its various molecular targets and properties has been under clinical investigation. Gathering all the high-quality clinical evidence to date, it appears that the effect of aspirin is still not delineated. Despite the large number of studies exploring aspirin's role in COVID-19 disease, the evidence is still premature. Almost all studies are retrospective, and many fail to consider baseline clinical status that might eventually alter the outcomes measured. More studies are needed to better define recommendations for clinical practice. The anti-inflammatory and anti-platelet properties of aspirin are appealing, yet future studies have to pledge to more objective designs. Multi-center placebo-controlled high-quality randomized clinical trials with plainly outlined baseline characteristics and outcomes are urgently needed to evaluate the efficacy of aspirin.

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