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Ramatroban for chemoprophylaxis and treatment of COVID-19: David takes on Goliath

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Abstract

Introduction: In COVID-19 pneumonia, there is a massive increase in fatty acid levels and lipid mediators with a predominance of cyclooxygenase metabolites, notably $TxB_2 \gg PGE_2 > PGD_2$ in the lungs, and 11-dehydro-TxB_2, a TxA_2 metabolite, in the systemic circulation. While TxA_2

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stimulates thromboxane prostanoid (TP) receptors, 11-dehydro-TxB_2 is a full agonist of DP2 (formerly known as the CRTh2) receptors for PGD_2. Anecdotal experience of using ramatroban, a dual receptor antagonist of the TxA_2/TP and PGD_2/DP2 receptors, demonstrated rapid symptomatic relief from acute respiratory distress and hypoxemia while avoiding hospitalization.

Areas covered: Evidence supporting the role of TxA_2/TP receptors and PGD_2/DP2 receptors in causing rapidly progressive lung injury associated with hypoxemia, a maladaptive immune response and thromboinflammation is discussed. An innovative perspective on the dual antagonism of TxA_2/TP and PGD_2/DP2 receptor signaling as a therapeutic approach in COVID-19 is presented. This paper examines ramatroban an anti-platelet, immunomodulator, and antifibrotic agent for acute and long-haul COVID-19.

Expert Opinion: Ramatroban, a dual blocker of TP and DP2 receptors, has demonstrated efficacy in animal models of respiratory dysfunction, atherosclerosis, thrombosis, and sepsis, as well as preliminary evidence for rapid relief from dyspnea and hypoxemia in COVID-19 pneumonia. Ramatroban merits investigation as a promising antithrombotic and immunomodulatory agent for chemoprophylaxis and treatment.

Keywords

Ramatroban; pharmacotherapy; COVID-19; long-haul COVID; thromboinflammation; thromboxane A_2; prostaglandin D_2; ARDS; interferon; lymphopenia; SARS-CoV-2; acute kidney injury; fibrosis; ischemia; platelets; immunomodulator; anti-platelet; IL-13; thrombosis; antithrombotic; cyclooxygenase (COX)

1. Introduction

The constellation of SARS-CoV-2 mediated, respiratory, epithelial, and vascular endothelial injuries, systemic inflammation, platelet activation, and platelet-leukocyte adhesion point to thromboxane A_2 (TxA_2) as a critical mediator of microvascular thrombosis in COVID-19 and potentially an important therapeutic target (Figure 1). TxA_2 and its metabolites, including 11-dehydro-TxB_2 are massively elevated in the bronchoalveolar lavage fluid, urine, and plasma in patients with COVID-19 [1–3]. These biomarkers correlated positively and significantly with microvascular thrombosis (D-dimers), hypoxia, need for mechanical ventilation, renal ischemia, duration of hospitalization and mortality [1–3]. The efficacy of low-dose aspirin, a cyclooxygenase-1 (COX-1) inhibitor, to treat COVID-19 remains questionable considering a marked increase in cyclooxygenase-2 (COX-2) expression in this disease [2,4]. Similarly, COX-2 inhibitors can enhance endothelial dysfunction and thromboinflammation by inhibiting prostacyclin synthesis [5]. Although thromboxane synthase (TS) inhibitors suppress TxA_2 formation, accumulation of the substrate PGH_2 stimulates thromboxane prostanoid (TP) receptors on platelets and endothelial cells, thereby inhibiting the anti-platelet action of TS inhibitors [6]. TP receptor antagonists block the activity of both TxA_2 and PGH_2 on platelets and endothelium but do not block TxA_2 production, leading to increased generation of 11-dehydro-TxB_2, a stable metabolite of TxA_2, and a potent agonist of prostaglandin D_2 receptor 2 (DP2) signaling [7] (Figure 2). Prostaglandin D_2 (PGD_2) has markedly increased in COVID lungs, and an increase in DP2 receptor expression has been reported in tissues in COVID-19 patients [8]. PGD_2/DP2

receptor signaling induces dysregulation of the innate and adaptive immune response to viral infections [2,9]. Thus, there is an unmet need for an orally bioavailable, potent, dual TxA_2/TP and PGD_2/DP2 receptor antagonist in COVID-19. Baynas® (ramatroban previously referred to as BAY u 3405; Bayer Yakuhin Ltd., Japan), a selective antagonist of the TP and DP2 receptors, has demonstrated efficacy in various animal models of respiratory inflammation, atherosclerosis, thrombosis, and sepsis and has been used to treat allergic rhinitis in Japan for over 20 years [10–12]. A review of the underlying mechanisms of inflammation associated with COVID-19 indicates that ramatroban can inhibit pro-inflammatory, pro-fibrotic, cardiovascular, and neuropsychiatric dysfunction as discussed hereunder.

2. Thromboinflammation in COVID-19

COVID-19, caused by SARS-CoV-2 infection, is associated with a prothrombotic state, which can present with thrombotic microangiopathy, pulmonary thrombosis, pedal acro-ischemia (‘COVID-toes’), arterial clots, strokes, cardiomyopathy, coronary, and systemic vasculitis, deep venous thrombosis, pulmonary embolism, and microvascular thrombosis in renal, cardiac, and brain vasculature [13–18]. Thrombotic microangiopathy is common, especially in children, and can lead to thrombocytopenia and bleeding [19]. Alveolar capillary microthrombi were 9 times more prevalent in patients with COVID-19 than in patients with influenza [13]. Necropsies have revealed inflammatory microvascular thrombi containing neutrophils, platelets, and neutrophil extracellular traps (NETs) in the pulmonary, hepatic, renal, and cardiac microvasculature as a hallmark of severe COVID-19 disease and the underlying cause of multi-organ failure [17,20,21]. Platelets have emerged as crucial effector cells, evidenced by platelet aggregation, adhesion, and spreading, followed by increased surface expression of P-selectin on platelets with circulating platelet-monocyte, platelet-neutrophil, and platelet-T cell aggregates [16,17,22,23] (Figure 1).

Platelet activation in COVID-19 is induced by endothelial damage [13,22]. SARS-CoV-2 infects endothelial cells, causing diffuse endothelialitis, intussusceptive angiogenesis, and impaired microcirculation in vascular beds [13,24,25]. Endothelialitis and pyroptosis lead to the release of microvesicles from infected endothelial cells, which activate leukocytes and platelets through surface interaction, receptor activation, cellular fusion, and the delivery of intra-vesicular cargo [25,26]. Elevated serum levels of soluble P-selectin, von Willebrand factor, soluble thrombomodulin, and soluble CD40L are evidence of endothelial cell injury and platelet activation in severe COVID-19 [22]. In addition, aberrant glycosylation of anti-SARS-CoV-2 spike immune complexes activates platelets and stimulates platelet thrombus formation on the von Willebrand factor [27]. This pathophysiology is reminiscent of endothelial-platelet-leukocyte activation and adhesion leading to a prothrombotic state described in sepsis where TxA_2 is a crucial mediator [28–30]. Similarly, platelet activation and thromboinflammation in COVID-19 appear to be fueled by a lipid mediator storm, as discussed below.

3. Role of thromboxane A_2 storm in COVID-19 associated thromboinflammation

TxA_2 is generated by platelets and modulates the functions of platelets and endothelial cells in a paracrine manner *via* the TP receptors [31] (Figure 1). Archambault and colleagues have measured eicosanoids in the bronchoalveolar lavage fluid (BALF) in 33 severely ill patients with COVID-19 within 2 hours of initiation of mechanical ventilation, compared with 25 healthy controls. Severe COVID-19 patients had marked increases in fatty acid levels as well as an accompanying inflammatory lipid storm with a predominance of arachidonic acid metabolites, notably $TxB_2 \gg PGE_2 > PGD_2$ [2]. The only other study that reported TxB_2 levels in BALF to the best of our knowledge was in atopic asthmatics [32]. A comparative analysis of TxB_2 levels in BALF across the two studies revealed an over 25-fold increase in BALF TxB_2 levels in COVID-19 patients compared to the levels in atopic asthmatics challenged with an allergen [32]. Plasma TxB_2 levels were also markedly increased in severe COVID-19 patients [16]. Urinary excretion of 11-dehydro-thromboxane B_2, a stable metabolite of TxA_2, is markedly increased in recently hospitalized patients with COVID-19; and was predictive of plasma D-dimer levels, renal ischemia, the need for mechanical ventilation and mortality [3].

TxA_2 mediates endothelial cell migration and angiogenesis in response to vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [33], the angiogenic growth factors that are markedly expressed in severe COVID-19 [13] (Figure 1). Additionally, TxA_2 also stimulates tissue factor (TF) expression on endothelial cells and monocytes [34–36] (Figure 1). TF expression by monocytes in hospitalized COVID-19 patients is associated with platelet-monocyte interaction, platelet activation, increased D-dimer levels, and finally, the need for invasive mechanical ventilation and subsequent mortality [16]. Furthermore, the TF is a high-affinity receptor for factors VII and VIIa and the primary activator of the coagulation cascade [37]. Thus, TF plays a central role in disseminated intravascular coagulopathy (DIC), and low-grade DIC is common in severe COVID-19 [38–40].

TxA_2 also stimulates P-selectin expression in platelets, and P-selectin plays a critical role in the initial adhesion and rolling of platelets and leukocytes to areas of injury and inflammation [29,41] (Figure 1). In addition, P-selectin plays a vital role in the formation of NETs, which is completely inhibited in P-selectin knockout mice or with P-selectin blockade [16,29,42]. In fact, in COVID-19 patients, NETs express TF, further increasing the thrombin-antithrombin activity [43]. On the other hand, induction of TF is inhibited by TxA_2/TP receptor antagonism in TNF-α stimulated endothelial cells and in lipopolysaccharide-stimulated human monocytes [34,36]. Notably, the TF expression is reduced by inhibiting COX-2, while inhibition of COX-1 is ineffective [34,44].

COX-1 is a constitutive isozyme found in most tissues and is involved in producing prostaglandins that regulate cellular housekeeping functions, including gastric cytoprotection, vascular homeostasis, platelet aggregation, and kidney function [35]. By comparison, COX-2 is preferentially expressed in first, kidney macula densa and controls renin secretion [45], and second, inflamed tissues or following exposure to growth factors,

cytokines, and other mediators of inflammation [35], which are elevated during SARS-CoV-2 infection [46–49]. Additionally, SARS-CoV-2 induces COX-2 expression in various cell lines and tissues *in vitro* and *in vivo*, including cardiomyocytes, Calu-3 and A549 lung cancer cell lines, ciliated lung cells, primary human bronchial epithelial cells, human ACE2-expressing mouse lungs, and living human lung slices [4,50,51]. COX-2 expression is elevated (>4-fold) in SARS-CoV-2 infected Calu-3 cells and human lung slices [4]. RNA-seq analysis of human-induced pluripotent stem cell-derived cardiomyocyte cells infected with SARS-CoV-2 versus uninfected controls revealed that COX-2 gene was significantly induced (>50-fold) in infected cardiomyocytes (Prof. Srinivasa T. Reddy, UCLA, personal communication following analysis of the supplemental material in reference 51) [51], and highly correlated with upregulation of pro-inflammatory genes and signaling pathways, including interferon, IL-1β, and NF-κB, all of which are significantly upregulated in COVID-19 [52]. Additionally, phospholipase A_2 is upregulated in severe COVID-19 [13,23], suggesting that the marked increase in plasma TxA_2 levels and TF in severe COVID-19 may, at least in part, be mediated by COX-2 [44,53]. Interestingly, SARS-CoV-2 infection-induced S1-ACE2 receptor interaction leads to NF-κB signaling in various cells [54,55]. NF-κB activates COX-2 expression and TxA_2 generation [56]. Furthermore, the nucleocapsid protein of SARS-CoV-2 binds to the Mannan-binding lectin-associated serine protease-2 (MASP-2), the lectin pathway's effector enzyme, resulting in a complement activation [18]. Lung tissue from deceased COVID-19 patients exhibits complement deposition including MASP-2, complement factor 4d (C4d), and C5b-9 (i.e. the membrane attack complex) [57,58]. Complement activation is a well-known driver of IL-1α production, a predictor of disease severity, and lung injury [59]. IL-1α is also released from necrotic and pyroptotic cells, including pneumocytes and endothelial cells, the primary sites of SARS-CoV-2 infection [60]. IL-1α triggers the expression of procoagulant and pro-inflammatory molecules, including COX-2 and thromboxane synthase, but not COX-1 [61,62].

In addition to the increased generation of TxA_2 and PGD2, SARS-CoV-2 infection inhibits the production of PG-degrading enzyme 15-hydroxyprostaglandin-dehydrogenase (15-PGDH) by about 90% [4]. 15-PGDH is the main enzyme for lipid mediator catabolism [4]. 15-PGDH can act on a wide variety of prostaglandin substrates with a high affinity for PGE2, $PGF_{2\alpha}$, PGI_2, and 6-keto $PGF_{1\alpha}$, and low affinity for PGD_2 and TxB_2 [63,64]. Thus, both COX-2 expression and 15-PGDH suppression appear to promote the lipid mediator storm in severe COVID-19 (Figure 3).

The thromboxane storm in COVID-19 may be partly fueled by a massive increase in megakaryocytes in the vascular beds of the lungs, heart, kidneys, and brain [20,65]. Extravascular spaces of the lungs comprise populations of mature and immature megakaryocytes [66]. They are a primary site of platelet biogenesis, accounting for approximately 50% of the total platelet production or about 10 million platelets per hour [67]. IL-6, which is markedly increased in severe COVID-19 [66], stimulates megakaryopoiesis [68,69]. COX-2 expression is induced during human megakaryopoiesis and characterizes newly formed platelets [70]. While in healthy controls, <10% of circulating platelets express COX-2, in patients with stimulated platelet generation, up to 60% of the platelets express COX-2 [70]. Generation of TxA_2 by platelets is markedly

suppressed by COX-2 inhibition in patients with increased megakaryopoiesis *versus* healthy subjects [70].

TP receptor activation by massively elevated levels of TxA_2 (and possibly isoprostanes, which also activate TP receptors) may be further compounded by increased expression of TP receptors. COX-2, thromboxane synthase, and TP receptor expression are markedly elevated in the obese [71–73]. In the intima of atherosclerotic coronary arteries, the TP receptor density increased about 3-fold [74]. We postulate the role of augmentation of TxA_2/TP receptor signaling with underlying obesity or coronary artery disease as a potential mechanism for the increased morbidity and mortality in COVID-19 patients with these comorbidities.

4. Thromboxane A_2 as a target for treating ARDS and thromboinflammation in COVID-19

TP receptor signaling leads to constriction of intrapulmonary veins and small airways with 10-fold higher potency and a greater reduction in luminal area than intrapulmonary arteries [75]. High local concentrations of TxA_2 in the lungs can effectively shut down pulmonary venous blood flow, increase microvascular pressure and permeability, and force fluid and plasma proteins into alveoli [75,76]. TP receptor antagonists are effective in the treatment of ARDS [77]. This therapeutic effect is thought to be secondary to inhibition of TxA_2/TP receptor-induced contraction of pulmonary veins, thereby relieving transcapillary pressure gradient across pulmonary capillaries, and transudation of fluid from the vascular compartment into the alveoli [75,76]. TP receptor antagonism also inhibits the pulmonary hypertension induced by PGB_2, an end product of PGE_2 metabolism [78]. TP receptor antagonism with ramatroban was previously reported to decrease pulmonary capillary pressure by selectively reducing post-capillary resistance in patients with acute lung injury [79]. This is consistent with rapid improvement in both respiratory distress and hypoxemia in a small case series of four consecutive COVID-19 patients with worsening respiratory distress and hypoxemia who were treated with ramatroban, thereby avoiding hospitalization and promoting recovery from the acute disease [76].

TxA_2 has been proposed as a target in COVID-19 for its role in thromboinflammation and microvascular thrombosis [80]. However, many of the customary therapies ultimately prove ineffective. Aspirin, for example, inhibits thromboxane generation by irreversibly inhibiting both COX enzymes (COX-1 ⩾ COX-2), preventing prostaglandin production by cells until a new enzyme is produced [81]. Low doses of aspirin, typically 75 to 81 mg/day, are sufficient to irreversibly acetylate serine 530 of COX-1 but have little effect on COX-2 [81]. Furthermore, aspirin is rapidly deacylated in the liver, such that the systemic concentrations of aspirin are too low to have any significant effects on thromboxane synthesis in tissues [82]. However, even low doses of aspirin sufficiently increase plasma concentrations in the portal vein so as to almost completely inhibit thromboxane generation in platelets as their transit through the portal circulation [83]. Therefore, aspirin has limitations as a treatment for COVID-19 since there is a massive generation of thromboxane in the tissues, especially the lungs which cannot be inhibited by low-dose aspirin [2]. This is consistent

with the absence of any significant benefit seen in hospitalized COVID-19 patients in the RECOVERY trial [84]. Furthermore, failure of aspirin may be partly secondary to aspirin resistance in the obese and elderly, which is characterized by unattenuated thromboxane production derived from elevated cytosolic phospholipase A_2 and COX-2 expression [84–87]. Similarly, inhibition of COX-2 increases the risk of cardiovascular events and is therefore not advisable in a prothrombotic disease such as COVID-19 [88–90]. Meanwhile, early use of aspirin to inhibit platelet TxA_2 synthesis has been proposed in COVID-19, but remains to be examined [91,92]. Aspirin has also been proposed to reduce the thrombotic complications from COVID-19 vaccination and stimulate the antibody response to COVID-19 vaccination as has been demonstrated for flu vaccine [91–95]. Thromboxane synthase inhibitors have limitations with continued PGH_2 and F2-isoprostane mediated TP receptor activation [6]. Anticoagulant agents, such as heparin and oral anticoagulants, are commonly used in COVID-19. However, these agents do not address the lipid mediator storm in the tissues that contributes to end organ failure, including ARDS due to the maladaptive hemodynamic, thromboinflammatory and immunomodulatory effects. Moreover, antithrombotic agents are associated with an increased bleeding risk [96,97]. Therefore, the early administration of well-tolerated TP receptor antagonists has been proposed to limit progression to severe COVID-19 by preventing ARDS and platelet-mediated thrombotic complications [76,92,98].

In summary, TxA_2/TP receptor signaling presents a therapeutic target in COVID-19 and long-haul COVID given its crucial role in the pathogenesis of thromboinflammation in this disease [5].

5. Role of thromboxane A_2 and prostaglandin D_2 in COVID-19-associated maladaptive innate and adaptive immune responses

In severe COVID-19, the levels of PGD_2 are significantly increased in the BALF [2], and plasma PGD_2 levels are increased about 5-fold in hospitalized COVID-19 patients (Prof. Srinivasa T. Reddy, UCLA; personal communication). PGD_2 is the most abundant prostanoid in the mammalian brain [99] and exerts its main functions through two receptors, DP1 and DP2 (also identified as CRTh2) [100]. DPr1 receptor stimulation increases intracellular cAMP and mediates first, anti-inflammatory effects by inhibition of cell migration and eosinophil apoptosis, and second, improvement of perfusion by relaxation of smooth muscle, vasodilatation, and antiplatelet actions [101]. In a model of bleomycin-induced acute lung injury, DPr1 deficiency, or inhibition exacerbated neutrophil infiltration, bronchoalveolar permeability and lung inflammation while inducing thymic atrophy and reducing survival [102].

5.1. Maladaptive type 2 immune response and immunosuppression in COVID-19 (Fig. 3)

DP2 receptor activation induces various pro-inflammatory downstream effects, which significantly contribute to the recruitment, activation, and/or migration of Th2 cells, ILC2, and eosinophils [103]. In severe COVID-19, the immune response is disproportionately shifted from a Th1 to a Th2 response characterized by an increase in plasma levels of type 2 cytokines produced by Th2 cells, including IL-4, IL-5 and IL-13 [104–106].

The effectors for PGD_2/DP2 receptor mediated Th2 immune response are eosinophils, basophils, mastocytes, and B cells (humoral immunity), and these are consistently elevated in COVID-19 [105]. Notably, PGD_2/DP2 receptor mediated Th2 immune responses are classically directed against extracellular non-phagocytosable pathogens, for instance, helminths [103,107,108]. Interestingly, IL-13 increases hyaluronan accumulation in mouse lungs [109] and is universally correlated with ARDS, acute kidney injury (AKI), and mortality [110], as well as a need for mechanical ventilation in COVID-19. IL-13 is also known to upregulate monocyte-macrophage-derived suppressor cells (MDSCs), which play a role in immune suppression and lymphopenia [111–113]. Th2 mediated inflammation and Th2 cytokines, especially IL-13 in COVID-19, implicate a critical role for PGD_2/DP2 receptors in disease severity.

Lymphopenia is one of the characteristic features of COVID-19 in adults and a predictor of disease severity [114] (Figure 3). Lymphopenia in COVID-19 may be caused by a massive expansion of MDSCs, with MDSCs populating up to 90% of the total circulating mononuclear cells in patients with severe disease and up to 25% in the patients with mild disease; the frequency decreasing with recovery [115]. Therefore, PGD_2/DP2 receptor mediated type 2 inflammation may contribute to lymphopenia and immunosuppression in COVID-19.

5.2. Suppressed and dysregulated innate immune responses in COVID-19 regarding suppression of interferon-λ and natural killer cell-mediated antiviral defenses

Type III interferons (IFN-λ) serve as the first line of defense against viral invasion at mucosal surfaces [116]. *In vitro, ex-vivo*, animal studies, and studies on SARS-CoV-2 infected patients have consistently demonstrated suppression of type III interferon (IFN-λ) expression in the upper respiratory tract (nose, mouth, and throat). A suppressed IFN-λ response appears to play a vital role in propagating SARS-CoV-2 to the lungs, thereby causing severe disease [117]. Zanoni and colleagues have reported that in patients <70 years of age, type III IFN expression is inversely associated with SARS-CoV-2 viral load and disease progression [118]. Furthermore, COVID-19 patients ≥70 years of age have lower type III IFN expression than younger patients, possibly contributing to increased morbidity and mortality in the elderly [118]. Moreover, IFN-λ1 was uniquely capable of inducing potent anti-SARS-CoV-2 interferon stimulating gene (ISG) expression in mild COVID-19, which was significantly decreased in the upper and lower airways of critically ill patients [118]. While the IFN-λ response is suppressed in severe COVID-19, it is preserved in patients with influenza of similar severity [119].

The mechanism of IFN-λ suppression in COVID-19 remains unclear. We postulate that PGD_2/DP2 receptor signaling plays a key role in the suppression of IFN-λ response in the upper respiratory tract during SARS-CoV-2 infection. In a neonatal mouse model of severe respiratory syncytial virus-induced bronchiolitis, treatment with a DP2 receptor antagonist decreased viral load and improved morbidity by upregulating interferon (IFN-λ expression [9,92]. It has been proposed that DP2 receptor antagonism may similarly promote antiviral immunity against early SARS-CoV-2 infection by restoring IFN-λ expression [92].

Interestingly, early, untimely TGF-β responses in SARS-CoV −2 infection limit the antiviral function of natural killer (NK) cells [120]. Platelets contain 40 to 100 times more TGF-β1 than other cells, and rapidly release TGF-β1 upon activation [121]. Ramatroban inhibits the release of TGF-β from platelets by blocking TxA_2 receptors [122]. Therefore, in COVID-19, ramatroban holds the potential for restoring NK cell antiviral function by inhibiting TGF-β release.

5.3. Crosstalk between thromboxane A_2 and PGD_2/DP2 receptor signaling

TxA_2 is short-lived and very rapidly transformed nonenzymatically in an aqueous solution to TxB_2. TxB_2 is further metabolized enzymatically into a series of compounds, of which 11-dehydro-TxB_2 is the major product found both in plasma and urine [7]. Interestingly, 11-dehydro-TxB_2 is a full agonist of the D-prostanoid receptor 2 (DP2) for prostaglandin D_2 (PGD_2) [7] (Figure 2). Furthermore, both PGD_2 and its metabolite, 9α11β-PGF_2, show similar potency for the TP receptor in the guinea pig aorta [123], and 9α11β-PGF_2 is a full agonist of the TP receptor in the human bronchus *in vitro* [124]. Therefore, selective antagonism of the DP2 receptor may exacerbate TP receptor mediated activity, similarly, selective antagonism of TP receptors may exacerbate PGD_2/DP2 receptor signaling (Figure 2).

The effects of PGD_2/DP2 receptor signaling mediated Th2 cytokines on the upper and lower respiratory tracts are further potentiated by TxA_2 produced by platelets, mast cells, and eosinophils. As a result, TxA_2 induces vasoconstriction and bronchoconstriction while increasing vascular permeability and airway hypersensitivity and is therefore, involved in the pathogenesis of allergic rhinitis and asthma [10,125].

The increased morbidity and mortality from COVID-19 observed in the elderly and the obese, may be related to increased generation of TxA_2 and PGD_2 in the elderly, and by adipose tissue in the obese [72,126–128]. This is clinically recognized as aspirin resistance in the obese and the elderly [86,87,129].

6. Ramatroban, a novel anti-platelet, immunomodulator, and antifibrotic agent for the treatment of acute and long-haul COVID-19?

Early in the course of the pandemic, TP receptor antagonists, such as picotamide, were proposed as a treatment for COVID-19 [130]. Several investigators have proposed that blocking the deleterious effects of TxA_2 and PGD_2 with a dual TP/DP2 receptor antagonist such as ramatroban might be beneficial in COVID-19 [2,80,131–133]. Ramatroban has been approved and safely used to treat allergic rhinitis in Japan since 2000 [10].

6.1. Mechanism of action of ramatroban with reference to COVID-19

Ramatroban is hypothesized to relieve lung edema and ARDS rapidly due to its beneficial hemodynamic effects on the pulmonary circulation as discussed above. If so, these benefits may extend to COVID-19, which is characterized by thromboinflammation and microvascular thrombosis associated with platelet activation that amplifies endotheliopathy [134]; monocyte activation with inflammatory type 1 macrophage (M1) phenotype [135];

and neutrophil activation with release of NETs [17,136]. A massive increase in lung and systemic thromboxane synthesized by endothelial cells, macrophages, and neutrophils in COVID-19 is a likely promoter of platelet activation induced directly by the spike protein of SARS-CoV-2 [2,16,135]. As a TP receptor antagonist, ramatroban is 100 times more potent than aspirin in inhibiting platelet aggregation and P-selectin expression [10,122]. When human platelet-rich plasma was stimulated by ADP, ramatroban inhibited the release of TxA_2, P-selectin, and TGF-β1 from platelets comparably to aspirin, but at a 1/100–1000th dose [122].

Ramatroban improves vascular responsiveness, while inhibiting endothelial surface expression of ICAM-1 and VCAM-1, inhibiting MCP-1 expression in response to TNF-α or platelet-activating factor, and inhibiting macrophage infiltration [10] (Table 1). In a rat model of endotoxic shock, ramatroban prevented hypotension, reduced plasma TNF-α levels by over 90%, and markedly reduced myeloperoxidase levels in lungs, ileum, and heart, suggesting end organ protection by mitigating TxA_2-mediated platelet-polymorphonuclear leukocyte activation. Ramatroban improved survival by 45% in endotoxic shock rats [137] (Table 2). In rats with splanchnic artery ischemia-reperfusion injury, the plasma levels of TxB_2 were increased about 7-fold [138]. Interestingly, ramatroban restored phagocytic function of peritoneal macrophages partially, inhibited plasma myocardial depressant factor activity about 50%, inhibited tissue infiltration by neutrophils, as measured by a decline in ilium myeloperoxidase activity >50%, reduced lung myeloperoxidase activity >80%; and prevented hypotension while improving survival [138] (Table 2). Notably, plasma myeloperoxidase is significantly increased in COVID-19 and is abundant in NETs, and regulates NET formation via synergy with neutrophil elastase [43,139]. Therefore, ramatroban is remarkably effective in both endotoxin- and ischemia-reperfusion injury-induced shock states, which share common pathogenetic mechanisms with severe COVID-19 [140]. Moreover, ramatroban prevented occlusive arterial thrombosis in response to vessel wall injury [141]. Infusion of ramatroban after coronary artery occlusion in dogs reduced myocardial infarct expansion by 65% and suppressed reperfusion arrhythmias [142].

COVID-19 is associated with complement-mediated thrombotic microangiopathy and hemolysis, especially in children [19]. Upon release, the reduced heme is rapidly and spontaneously oxidized in the blood into its ferric (Fe^{3+}) form, hemin, with increased levels observed in hemolytic diseases [155]. Hemin activates platelets by serving as a ligand for C-type-lectin-like receptor 2 (CLEC2) [155]. Upon activation, the CLEC2 receptor undergoes tyrosine phosphorylation mediated by TxA_2 [147]. This leads to downstream phosphorylation of spleen tyrosine kinase and phospholipase γ2, potentiated by TxA_2 [147]. This cooperation between CLEC2 and TxA_2 signaling is critical for platelet activation in hemolytic states [147]. Platelet activation leads to the release of exosomes and microvesicles, which further activate CLEC5A on neutrophils and TLR2 on macrophages, thereby inducing NET formation and pro-inflammatory cytokine release [156]. The potentiation of CLEC2 signaling by TxA_2 was abolished by 10 μM ramatroban; by comparison, 1 mM aspirin was largely ineffective [147] (Table 1). Therefore, ramatroban may be more effective than aspirin in abrogating TxA_2-dependent CLEC2 signaling, platelet activation, and thromboinflammation in COVID-19 associated thrombotic microangiopathy.

Sugimoto *et al.* have reported that ramatroban has a potent and selective antagonist activity against the DP2 receptor while sparing the DP1 receptor [157]. The IC_{50} of ramatroban for inhibiting IL-4, IL-5, and IL-13 production induced by PGD_2 (100 nM) is 103, 182, and 118 nM, respectively [112]. By comparison, the typical adult dose of 75 mg twice a day achieves an average plasma concentration of about 240 nM [146], roughly 1.5-2-fold the concentration required for effective inhibition of type 2 cytokines, including IL-13, the key biomarker of COVID-19 severity. Ramatroban was shown to reduce dermal neutrophilic and eosinophilic infiltrate in a Th2-dependent murine model of fluorescein isothiocyanate-induced contact hypersensitivity that closely parallels the acute inflammatory pathology of human atopic dermatitis [158]. Oral administration of ramatroban inhibited bronchoconstriction induced by TxA_2, PGD_2, $PGF_{2\alpha}$, and inhaled LTC_4 and LTD_4 [159]. Ramatroban inhibited asthma pathology *in vivo* by reducing peribronchial eosinophilia and mucus cell hyperplasia [153]. In experimental allergic reactions, ramatroban inhibited antigen-induced respiratory resistance in Guinea pigs, allergen-induced biphasic increase in respiratory resistance and airway inflammation in mice, and antibody-mediated skin reactions in mice [160]. Thus, PGD_2/DP2 receptor antagonism with ramatroban may correct the maladaptive immune responses characterized by polarization to Th2 ≫ Th1 response leading to lymphopenia during severe SARS-CoV-2 infection (Figure 3).

6.2. Ramatroban to mitigate end organ injury and long-haul COVID

In addition to platelet activation and thromboinflammation, SARS-CoV-2 induces endothelial dysfunction, vasoconstriction, and ischemia-reperfusion injury leading to pulmonary, cardiac, hepatic and renal end organ injury [161]. As described previously, ramatroban reduces ischemia-reperfusion injury to the organs, in part by mitigating thrombinflammation [138], and by enhancing the generation of vasodilatory nitric oxide [162], thereby promoting vasoprotection.

Ramatroban may also prevent fibrosis, a characteristic feature of long-haul COVID [163]. TGF-β is elevated in COVID-19 [120]. TGF-β is known to mediate hepatic, renal, pulmonary and cardiac fibrosis in various animal models [164–167]. Ramatroban inhibits platelet TGF-β1 release [122]. Moreover, the pro-apoptotic and pro-fibrotic effects of PGD_2/DP2 receptor signaling in the lungs, heart, liver, and kidneys may mediate acute lung injury, myocardial dysfunction, and acute kidney injury in COVID-19 [12,168–170]. Therefore, ramatroban may exert an antifibrotic effect in COVID-19 and long-haul COVID by blocking TGF-β1- and PGD_2/DP2 receptor-induced fibrosis.

Although there is currently no animal model of SARS-CoV-2-induced fibrosis to date, pulmonary pathology in COVID-19 most closely resembles an animal model of silicosis, which exhibits massive increases in lung PGD_2 and TxA_2 associated with lung inflammation and fibrosis [12] (Table 2). In this silicosis model, ramatroban reduced macrophage, lymphocyte and neutrophil infiltration of the lungs while inhibiting TNF-α, IL-6, IL-1β, IL-18 and NLRP3 activation, thereby reducing inflammation, fibrosis and cardiopulmonary dysfunction [12] (Table 2). Moreover, in a model of premature, age-related heart failure [152], PG-degrading hydroxyprostaglandin-dehydrogenase-15, the primary enzyme for lipid mediator catabolism, was significantly elevated, similar to its

elevation in COVID-19 patients [4]. Subsequent elevation of PGD_2 secretion was associated with enhanced adipocyte accumulation in aged male mouse hearts and young male mice with cardiomyocyte-specific STAT3 deficiency. Conversely, DP2 receptor antagonism with ramatroban *in vivo* increased EZH2 expression and reduced ZFP423 expression in cardiomyocyte progenitor cells, thereby abrogating adipocyte differentiation in STAT3 deficiency and promoting cardioprotection. Therefore, the vasoprotective and antifibrotic effects of ramatroban have the potential to prevent end organ injury and transition to severe disease that has been described as long-haul COVID as discussed below.

Ramatroban may address persisting sequelae symptoms following recovery of acute illness referred to as post-acute sequelae of SARS-CoV-2 infection or 'long-haul' COVID (Figure 3). The lipid mediator storm during acute COVID-19 coupled with the neurologic, immunologic, and prothrombotic phenotype of long-haul COVID raises the specter of prolonged thromboinflammation and sustained elevation in lipid mediators fueling long-haul COVID syndrome. Common characteristics of long-haul COVID include neuropsychiatric manifestations including 'brain fog,' anxiety or depression, fatigue, and problems with mobility, dyspnea due to lung fibrosis and lung diffusion impairment, and microvascular thrombosis persisting for >4 months in about 25% of the patients [171,172]. To date, there has been no animal model for long-haul COVID. However, in well-established models (Table 2), including chronic corticosterone-, lipopolysaccharide-, and tumor-induced pathologically relevant depression models, elevations in PGD_2 mediate depression-like behavior, while ramatroban restores object exploration and social interaction [173]. Therefore, ramatroban is a promising candidate for chemoprophylaxis and treatment of long-haul COVID sequelae symptoms, including neuropsychiatric manifestations and end organ injury and fibrosis involving lungs, kidneys, heart, and liver (Figure 3).

Concisely summarized, due to its immunomodulatory, antithrombotic, anti-inflammatory, and antifibrotic action, ramatroban is likely to provide a therapeutic benefit at all stages of COVID-19 disease. The acute relief of dyspnea and hypoxemia following ramatroban administration in four patients with moderate-to-severe COVID-19 supports a compelling need for clinical trials of ramatroban for hospitalized, non-ICU COVID-19 patients [76].

7. Safety profile of ramatroban

The intravenous LD_{50} values in mice and rabbits were >600 and >100 mg/kg, respectively, while no dogs died with an intravenous dose of 250 mg/kg [10]. In a 12-months toxicity study of dogs, no toxicologically important changes were observed in any dog given up to 30 mg/kg/day of ramatroban. In this study, the plasma concentration of ramatroban in animals at 2 h after oral administration of 30 mg/kg of the drug was between 11.9 and 32.7 mg/mL, while C_{max} in healthy adult male volunteers given 75 mg of ramatroban twice daily (usual clinical dose) was about 0.4 mg/mL. Accordingly, the doses tested were judged to be sufficiently high to indicate the clinical safety of ramatroban in humans.

Ramatroban is metabolized in the liver by the cytochrome P450 enzyme, CYP3A4 through acylation by glucuronic acid (primary) and hydroxylation (secondary). Over 90% of ramatroban is excreted by the hepatobiliary route, and <10% is excreted renally, unchanged,

or as metabolites, indicating a high probability of effective excretion of ramatroban in COVID-19 patients with renal failure. The cytochrome P450 inhibition of ramatroban is not significant considering that the inhibition constant for CYP2C9 is 25 μM, which is 25 times greater than the peak blood level achieved with 75 mg of ramatroban [10]. The potential for pharmacodynamic and pharmacokinetic interactions between ramatroban and other drugs used in severe COVID-19 patients remains to be elucidated. Several potential COVID-19 drugs, which are, at least in part, metabolized by or inhibit CYP3A4 include remdesivir, lopinavir/ritonavir, umifenovir, ivermectin, atazanavir, ruxolitinib, baricitinib, imatinib, and fluvoxamine [174]. Further studies are needed to establish the drug-drug interactions between ramatroban and these drugs.

Ramatroban is generally safe when taken in the prescribed dosage range. Pivotal trials and post-marketing surveys (n = 4,443) demonstrated the following *adverse events*: first, bleeding in 0.19% (nose bleeds, 0.07%; gingival bleeding, 0.05%; subcutaneous bleeding, 0.02%; and hypermenorrhea, 0.05%); second, neuropsychiatric complications in 1.1% (drowsiness and headache/heaviness in head in about 0.5% each); and third, elevations in liver function tests in 2.2% (ALT/AST/gamma GT, <1%; alkaline phosphatase, <0.5%; LDH, <0.5%; bilirubin, <0.2%). Prolongation of bleeding time is the expected pharmacological action of the drug.

8. Clinical efficacy of ramatroban

Several clinical trials, as summarized in the Japanese package insert of Baynas® (ramatroban) have described the safety and efficacy of ramatroban in allergic rhinitis and perennial nasal allergies [146] (Table 3). Ramatroban, 75 mg per day administered orally, reduces bronchial hyperresponsiveness to methacholine in asthmatic patients [175]. Ramatroban significantly reduces local eosinophilia and nasal mucosal swelling in allergic rhinitis. In a double-blind, randomized control trial of patients with allergic rhinitis, final overall improvement with ramatroban classified as ‘moderate improvement’ was found in 66.7% (186/279) patients. Furthermore, a study of 33 patients, with perennial nasal allergy taking ramatroban for 4 weeks revealed a significantly reduced degree of nasal congestion. In a similar study of 59 patients with moderate/severe perennial nasal allergy, ramatroban dose-dependently increased the overall improvement rate up to 72.7% and decreased nasal obstruction up to 90.9% with the typical adult dose of 75 mg BID. Moreover, a randomized parallel dose-response study of 251 patients with severe perennial nasal allergy and moderate nasal congestion revealed a significant relationship between ramatroban doses of sneezing and nasal discharge. A comparative test with terfenadine also confirmed the usefulness of ramatroban for perennial nasal allergy.

Ramatroban is available in two oral-dose forms, 50 or 75 mg tablets, to be taken twice daily. The usual adult oral dose of 75 mg twice a day achieves an average plasma concentration of about 0.1 mg/L or 240 nM, which is sufficient to both inhibit platelet activation (since the IC_{50} for human platelet aggregation is only about 30 nM) [10] and type 2 interleukin production (vide infra). Ramatroban exhibits surmountable binding to the TP receptor [176], and with a plasma half-life of about 2 hours, platelet-dependent hemostasis is unlikely to be continuously impaired with 75-mg doses given about 12 hours apart [10,146]. This

is advantageous in the event of bleeding complications reported in 5.6% of critically ill COVID-19 patients [177]. A recent case series reports rapid improvement of 4 patients with severe COVID-19 treated with ramatroban, leading to relief of respiratory distress and hypoxemia [76]. One patient in particular was a frail 87-year old lady with a past medical history of hypertension, stage-IV chronic kidney disease and myocardial infarction who subsequently developed severe COVID-19 and ARDS. Having failed treatment with remdesivir and corticosteroids, the patient was subsequently started on 37.5 mg ramatroban twice daily, leading to rapid improvement and complete recovery [76].

In patients with perennial allergic rhinitis, 4-week treatment with ramatroban significantly inhibited the increase in eosinophil counts in the nasal lavage fluid 30 minutes after allergen challenge and reduced eosinophil cationic protein levels after challenge [178]. Therefore, ramatroban has demonstrated clinical amelioration of allergic type 2 immune response that mirrors the maladaptive immune and inflammatory profile in COVID-19 [105,109]. Although numerous animal models demonstrate effective inhibition of platelet activation, vascular inflammation and fibrosis with ramatroban [11,12,137,138,149,154], the clinical antithrombotic and antifibrotic effects of ramatroban remains to be studied.

9. Expert opinion

Pharmacologic inhibition of COX-1 or COX-2 expression can prevent the generation of a plethora of pro- and anti-inflammatory lipid mediators. Low-dose aspirin mitigates the generation of TxA_2 by irreversible inactivation of the constitutive COX-1 but not the inducible COX-2 and has been shown to be ineffective as a treatment for severe COVID-19 [84]. A more definitive approach to prevent thromboinflammation in COVID-19 might be to directly block the prothrombotic effects of TxA_2. Although thromboxane synthase (TS) inhibitors suppress TxA_2 formation, accumulation of the substrate PGH_2 stimulates the TxA_2 prostanoid (TP) receptor on platelets and endothelium, thereby inhibiting the anti-platelet action of TS inhibitors [6]. Thus, this approach fails to meet the therapeutic needs of patients with severe COVID-19. Likewise, TP receptor antagonists block the activity of both TxA_2 and PGH_2 on platelets and endothelium. Still, they do not block TxA_2 production, leading to increased generation of 11-dehydro-thromboxane B_2, a stable metabolite of TxA_2 and a potent agonist of PGD_2/DP2 receptor signaling [7]. PGD_2/DP2 receptor signaling induces dysregulation of innate and adaptive immune responses in viral infections, including COVID-19.

In contrast to the shortcomings of therapeutic agents in the classes described above, ramatroban is an orally bioavailable, potent, dual TxA_2/TP and PGD_2/DP2 receptor antagonist, with demonstrated efficacy in a variety of animal models of respiratory inflammation, atherosclerosis, thrombosis, and sepsis [10]. The safety profile of ramatroban is well established, and the drug has been used in Japan over the past 20 years to treat allergic rhinitis [10].

Based on the mechanisms of action as outlined in Figure 3, it is reasonable to propose that ramatroban may serve as a multipronged approach for chemoprophylaxis and treatment of COVID-19. Inhibiting PGD_2/DP2 receptor signaling with ramatroban may

first limit the progression of SARS-CoV-2 infection and reduce the viral load by restoring the IFN-λ response and second, inhibit the ILC2/IL-13 mediated immunosuppression and lymphopenia, thereby restoring the host's viral immunity [2,80,92]. Ramatroban by inhibiting TxA_2/TP receptor signaling may mitigate first, thromboinflammation and resulting ischemia-reperfusion injury to organs and second, inhibit the pulmonary venous constriction that contributes to increased pulmonary capillary pressure and ARDS [75–77].

By addressing the lipid-mediator storm, ramatroban may address the great unmet need for safe, effective, and inexpensive therapeutics in the burgeoning number of patients with acute COVID-19 [76]. Starting early in the disease course, ramatroban could potentially prevent progression to severe COVID-19. As an antithrombotic and immunomodulator ramatroban could be considered in addition to the sequenced multidrug treatment of ambulatory and hospitalized COVID-19 patients. The authors have urged the inclusion of ramatroban in the Platform trials being conducted for COVID-19, the ACTIV trials by the National Institutes of Health, USA, the RECOVERY trial in the United Kingdom; and the SOLIDARITY trial by the World Health Organization. It is hoped that, in combination with an oral antiviral agent, ramatroban could further reduce the intensity and severity of symptoms and by that mechanism, reduce the risk of hospitalization and subsequent death [76]. Furthermore, the hemodynamic, antithrombotic, anti-apoptotic, and antifibrotic actions of dual TP and DP2 receptor antagonism with ramatroban may prevent end organ injury and progression to long-haul COVID. Thus, ramatroban merits further investigation as a promising antithrombotic and immunomodulatory agent for chemoprophylaxis and treatment of patients with COVID-19.

Declaration of Interest

AG and KCC have filed a patent on the use of ramatroban for COVID-19 and other indications.

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Abbreviations

TxA_2	thromboxane A_2
TxB_2	thromboxane B_2
TP	thromboxane prostanoid
PGD_2	prostaglandin D_2
DP2	prostaglandin D_2 receptor 2
DP1	prostaglandin D_2 receptor 1
DIC	disseminated intravascular coagulopathy
COX	cyclooxygenase

TF	tissue factor
NET	neutrophil extracellular trap
MCP-1	monocyte chemoattractant protein 1
VCAM-1	vascular cell adhesion molecule 1
ICAM-1	intercellular adhesion molecule 1
VEGF	vascular endothelial growth factor
bFGF	basic fibroblast growth factor
TNF-α	tumor necrosis factor-alpha
IL	interleukin
NF-κB	nuclear factor- κB
BALF	bronchoalveolar lavage fluid
Th1 and −2	T-helper cell type 1 and 2
15-PGDH	PG-degrading enzyme 15-hydroxyprostaglandin-dehydrogenase
AKI	acute kidney injury
ARDS	acute respiratory distress syndrome
COVID-19	coronavirus disease 2019
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
NK	natural killer

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ARTICLE HIGHLIGHTS

1. COVID-19 pneumonia exhibits a sustained extraordinary lung accumulation of lipid mediators, especially thromboxane A_2. Thromboxane A_2 metabolites are associated with respiratory failure and mortality in COVID-19.
2. Pulmonary hemodynamic changes in COVID-19 pneumonia, including post-capillary hypertension in the majority of patients, are consistent with thromboxane prostanoid (TP) receptor-dependent pulmonary venoconstriction leading to pulmonary edema and ARDS. TP receptor antagonism improves ventilation-perfusion matching and relieves hypoxemia.
3. COVID-19 associated thromboinflammation is consistent with thromboxane A_2-mediated platelet activation, platelet-monocyte and platelet-neutrophil interactions, and endotheliopathy leading to microvascular thrombosis, hypoxemia, and end organ damage.
4. A suppressed interferon-λ response in the upper respiratory tract is associated with the severity of COVID-19. PGD_2/DP2 receptor signaling suppresses interferon-λ expression, whereas the DP2 receptor antagonism stimulates interferon-λ expression and suppresses viral replication.
5. The adaptive immune response in COVID-19 is polarized toward a Th2 rather than a Th1 immune response inducing lymphopenia and immune suppression. PGD_2/DP2 receptor signaling supports a shift of Th2 ≫ Th1.
6. The anecdotal experience of using ramatroban, a dual receptor antagonist of the TxA_2/TP and PGD_2/DP2 receptors, in adult COVID-19 outpatients demonstrated rapid symptomatic relief from acute respiratory distress and hypoxemia while avoiding hospitalization. This approach merits further testing in randomized controlled clinical trials.

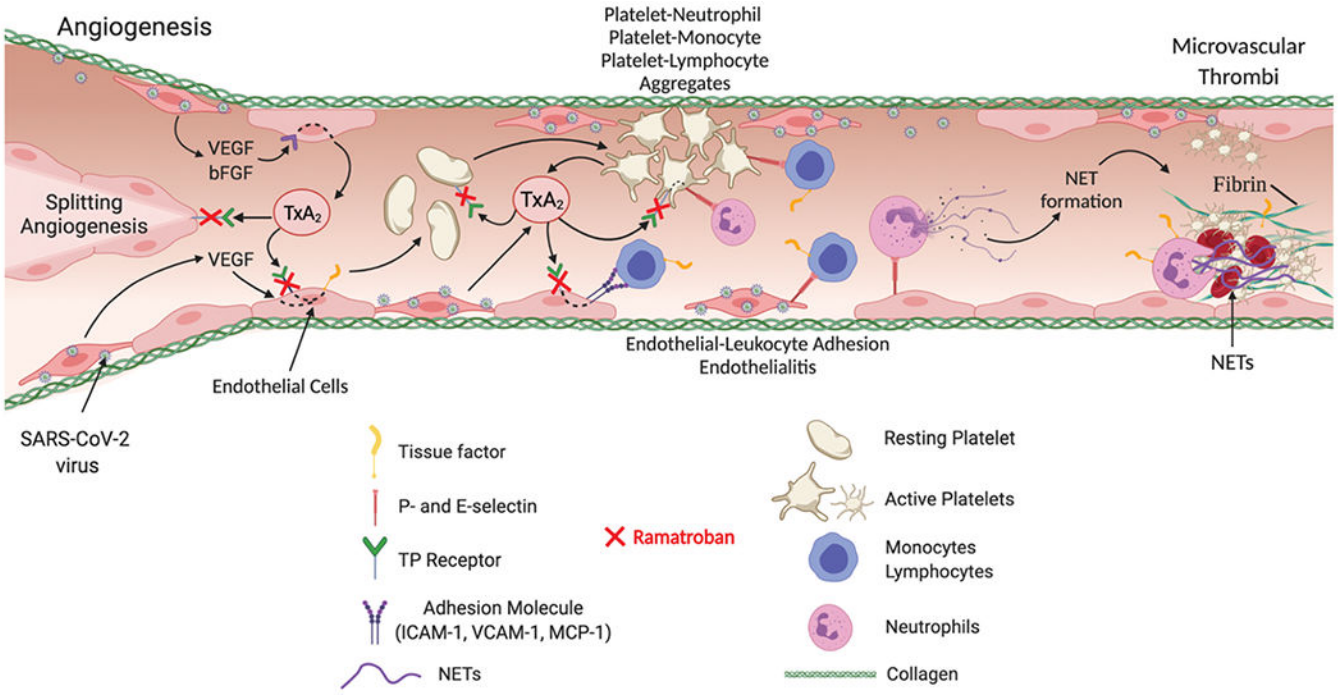

Figure 1. Thromboinflammatory dysregulation in COVID-19 and proposed mechanism of action of ramatroban as a thromboxane A_2 receptor antagonist in targeting the underlying pathogenetic mechanisms.

SARS-CoV-2 induced endothelial cell activation leads to cyclooxygenase (COX)-2 expression and thromboxane A_2 (TxA_2) generation. TxA_2 stimulation of the thromboxane prostanoid (TP) receptor on endothelial cells leads to surface expression of leukocyte adhesion molecules including ICAM-1, VCAM-1 and MCP-1, which promote recruitment and migration of monocytes, lymphocytes and neutrophils. TxA_2/TP receptor axis induces activation and P-selectin expression on platelets and endothelial cells, leading to monocyte and neutrophil activation. Activated monocytes and endothelial cells express tissue factor (TF) while activated neutrophils release neutrophil extracellular traps (NETs) expressing TF which contribute to the formation of inflammatory microvascular thrombi. Activated endothelial cells also release vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) which stimulate endothelial TF expression and TxA_2 release. In turn, TxA_2 stimulates endothelial TP receptor to promote endothelial cell migration and splitting angiogenesis in COVID-19. COX, cyclooxygenase; TxA_2, thromboxane A_2; TP, thromboxane prostanoid; MCP-1, monocyte chemoattractant protein 1; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TF, tissue factor; NETs; neutrophil extracellular traps; (created with BioRender.com).

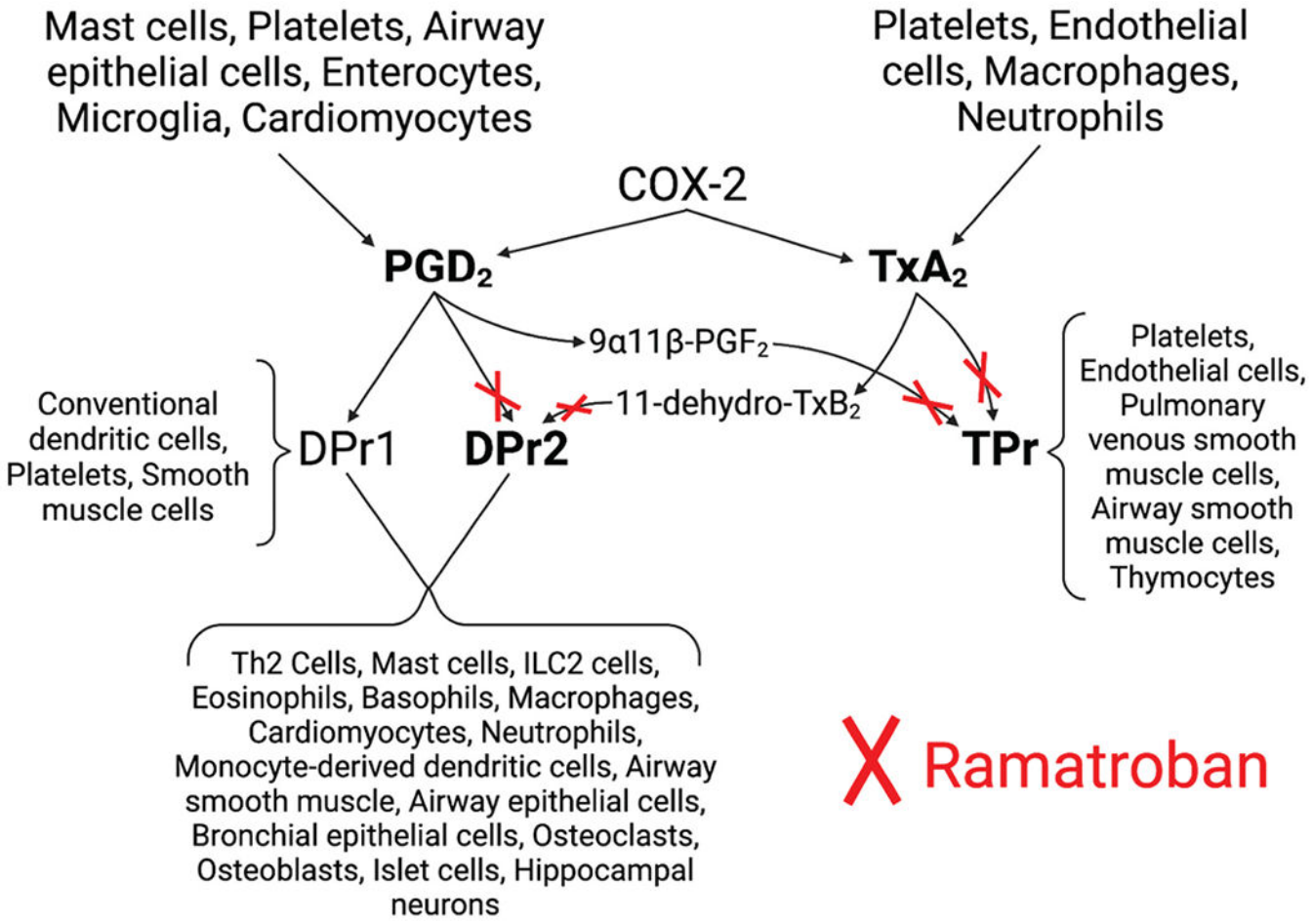

Figure 2. Crosstalk between PGD_2,TxA_2 and the targets of ramatroban:

COX-2 mediates downstream production of prostaglandin D_2 (PGD_2) and thromboxane A_2 (TxA_2). The stable metabolites of TxA_2 and PGD_2 are 11-dehydro-TxB_2 and 9α11β-PGF_2, respectively. 11-dehydro-TxB_2 and 9α11β-PGF_2 are agonists of the PGD_2/DP_2 and TP receptors, respectively. As a dual receptor antagonist of the DP2 and TP receptors, ramatroban blocks the pro-inflammatory and prothrombotic effects of PGD_2, TxA_2 and their respective metabolites while sparing the anti-inflammatory DP1 receptor. COX-2, cyclooxygenase-2; PGD_2, prostaglandin D_2; TxA_2, thromboxane A_2; 9α11β-PGF_2, 9α11β-prostaglandin F_2; 11-dehydro-TxB_2, 11-dehydro-thromboxane B_2; TPr, thromboxane prostanoid receptor; DPr2, prostaglandin D_2 receptor 2; DPr1, prostaglandin D_2 receptor 1. (created with BioRender.com).

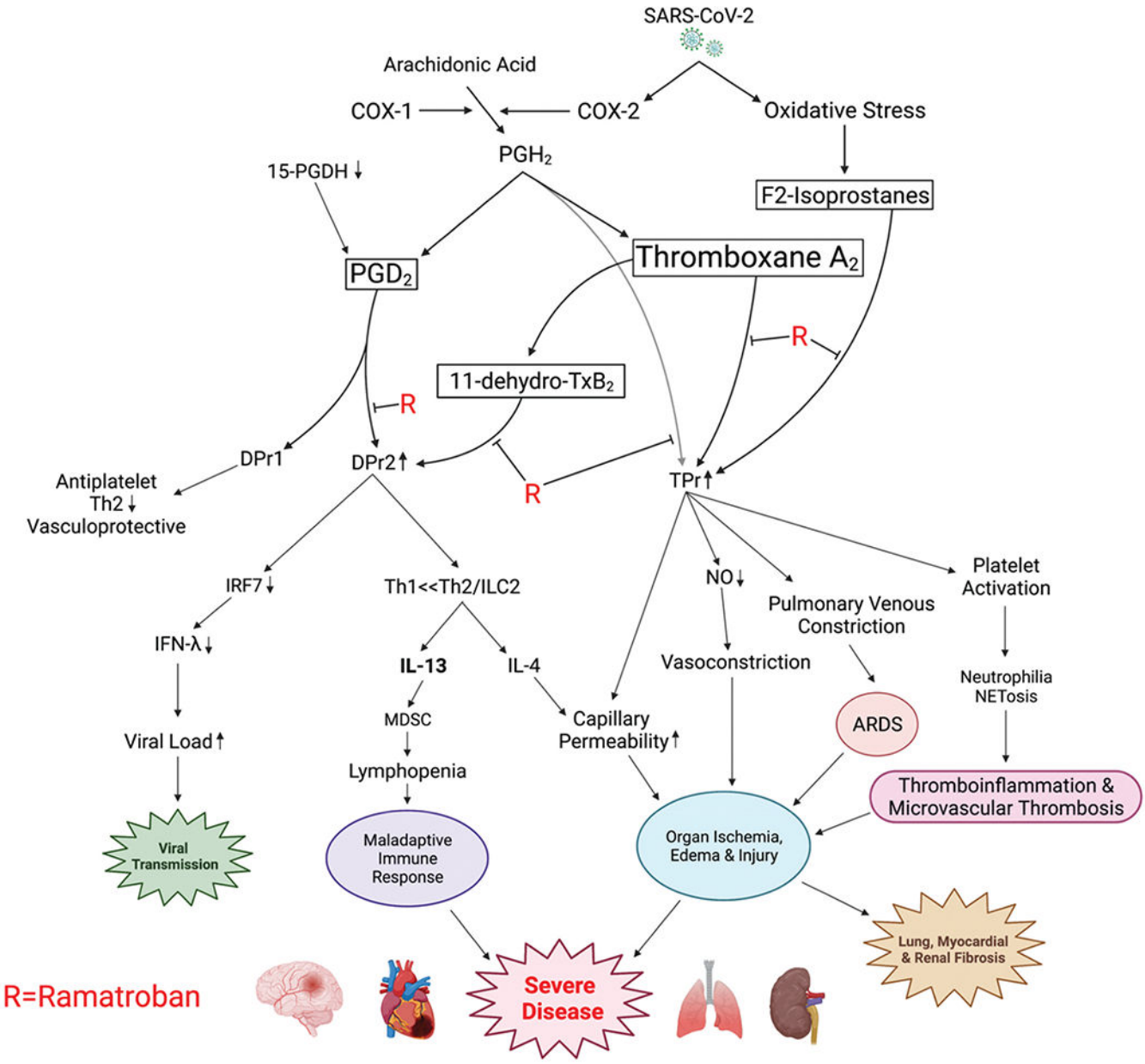

Figure 3.

Putative mechanisms of SARS-CoV-2 induced acute severe disease mediated by deleterious COX-2-derived lipid mediators including PGD_2 and TxA_2, which is mitigated by dual receptor antagonism of the PGD_2/DP2 and TxA_2/TP receptors with ramatroban. COX, cyclooxygenase; PGD_2, prostaglandin D_2; TxA_2, thromboxane A_2; PGH_2, prostaglandin H_2; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; 11-dehydro-TxB_2, 11-dehydro-thromboxane B_2; TPr, thromboxane prostanoid receptor; DPr2, prostaglandin D_2 receptor 2; DPr1, prostaglandin D_2 receptor 1; Th1 and −2, T-helper cell type 1 and 2; IL, interleukin; MDSC, myeloid-derived suppressor cells; ARDS, NO, nitric oxide; IRF7, interferon

regulatory factor 7; IFN, interferon; ARDS, acute respiratory distress syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. (created with BioRender.com).

Table 1.

Effect of ramatroban *in vitro.*

Model	Outcome
HEK 293 T cells expressing A160T variant *in vitro*	Basal Calcium mobilization 70% ↓****
[143]	IP_3 mobilization 50% ↓*
Human megakaryocytes expressing A160T variant *in vitro*	P-selectin expression on platelet like particles >30% ↓**
[143]	
Platelet Rich Plasma	Aggregation with 5 μM/L ADP 33% ↓*
[122].	sP-selectin >35% ↓NS TGF- β1 > 35% ↓NS TxB_2 > 35% ↓NS Platelet aggregatory threshold index 388% ↑* (ADP induced 50% pressure rate) Aggregation with 1 μg/mL collagen 65% ↓* sP-selectin >60% ↓* TGF-β1 > 60% ↓* TxB_2 > 60% ↓* Aggregation with 3 mM/L arachidonic acid 71% ↓* sP-selectin >65% ↓* TGF- β1 > 65% ↓* TxB_2 > 70% ↓*
Model	**Outcome**
Human Th2 cells stimulated with increasing concentration of PGD_2 for 4 hours *in vitro*	IL-4 (pg/ml) ~100% ↓*
[112]	IL-5 (↓g/ml) ~55% ↓* IL-13 (pg/ml) ~40% ↓*
[^{3}H]Sphingosine-labeled human platelets stimulated with protease-activated receptor-1-activating peptide *in vitro*	[^{3}H]Sphingosine 1 phosphate release ~195% ↓*
[144]	
Human umbilical vein endothelial cells (HUVEC)	TNF-α stimulation
[145]	MCP-1 mRNA/β-actin mRNA >65% ↓** MCP-1 (ng/mg protein) ~65% ↓** Platelet-activating factor stimulation MCP-1 mRNA/β-actin mRNA ~50% ↓** MCP-1 (ng/mg protein) ~45% ↓** U46619 stimulation MCP-1 mRNA/β-actin mRNA ~60% ↓** MCP-1 (ng/mg protein) ~80% ↓** Cell viability NS ^{35}S-methionine counts (dpm/pg protein) NS
U-46619 stimulated human microvascular endothelial cells	ICAM-1 100% ↓**
[146]	VCAM-1 80% ↓**
Non-aspirin treated human platelets	Rhodocytin & U46619 stimulation
[147]	Syk phosphorylation >90 ↓** PLCγ2 phosphorylation >90 ↓** CLEC2 signaling 100% ↓**

Model	Outcome
Guinea pig peripheral lung tissue [148]	U46619 or PGD_2/TP receptor signaling ^ contraction of pulmonary veins ≫ arteries ~50% ↓ *

NS = not significant;

* p < 0.05;

** p < 0.01;

*** p < 0.001;

**** p < 0.0001

^ the effect of PGD_2 was mediated by the TP receptor and not the DP1 or DP2 receptors

Table 2.

Effect of ramatroban in Animal Models.

Model	Effect of ramatroban
Splanchnic artery occlusion in male rats [138]	Restores survival rate (%) ↑***
	Survival time 100% ↑**
	Mean arterial pressure 50%↑***
	Myocardial depressant factor (U/ml) 50%↓**
	Ileum myeloperoxidase activity 60%↓*
	Lung myeloperoxidase activity 85%↓*
	Macrophage Phagocytosis 56%↑*
Endotoxin shock male rats [137]	Increases Survival Rate to 45% ↓*
	Systemic hypotension ↓*
	Phagocytosis 79% ↑***
	Serum TNF-α ↓*
	Ileum MPO activity 40% ↓*
	Heart MPO activity 56% ↓*
	Lung MPO activity 35% ↓*
Coronary artery occlusion and reperfusion rats [149]	Restores survival rate (%) ↑**
	Serum creatine phosphokinase 53% ↓**
	Pressure Rate Index 28% ↑*
	Necrotic/Area at risk (%) (wet heart weight) >50% ↓**
	Cardiac MPO Activity: Area at risk >50% ↓**
	Necrotic area >65% ↓**
Model	**Effect of ramatroban**
Unilateral hindlimb ischemia in the right femoral artery ligature of male mice [150]	VEGF-A protein content ~40% ↓*
	Microangiographic Score (IR/non-IR ratio). ~30% ↓*
	Capillary Density (nb/mm^2) >20% ↓*
	Foot Blood Perfusion (IR/non-IR ratio) ~20% ↓*
	Mac-3 positive cells ~25% ↓*
CRTH2$^{+/+}$ mice [151]	Lipopolysaccharide inoculation Social interaction ~260% ↑**
	Novel exploratory behavior ~230% ↑**
	Tumor inoculation Social interaction ~325% ↑**
	Novel exploratory behavior ~80% ↑*

Model	Effect of ramatroban
	Lipopolysaccharide induced c-Fos expression in the brain (6 hours) Nucleus of the solitary tract ~27% ↓^{NS}
	Bed nucleus of stria terminalis ~31% ↑^{NS}
	Hypothalamus paraventricular nucleus ~48% ↓*
	Central amygdala ~57% ↓*
Cardiac progenitor cells from STAT3 knockout young male mice	White adipocyte differentiation ↓ Enhancer of zeste homolog 2 (EZH2) expression ~50% ↑*
[152]	Zinc finger protein 423 (ZFP423) expression ~20% ↓*
48-hour PGD_2 treatment of human pluripotent stem cells (induced)	EZH2 expression ~90% ↑**
	ZFP423 expression ~80% ↓**
[152]	Oil Red O staining ~95% ↓**
	CCAAT/enhancer-binding protein alpha ~85% ↓**
Mice immunized with allergen (OVA) and challenged twice with inhalation of aerosolized OVA	Lung tissue eosinophils/0.1 mm^2 ~ 40% ↓*
[153]	Goblet cells (cells/mm basement membrane) ~30% ↓*
	BALF eosinophilia 60% ↓*
Model	**Effect of ramatroban**
Balloon injury of hypercholesterolemic Rabbits	Acetylcholine
[11]	Attenuation of vascular response 8-iso-prostaglandin$F_{2\alpha}$ Attenuation of vascular response
Balloon injury of hypercholesterolemic rabbits	MCP-1 gene expression in injured aortas ~82% ↓*
[154]	Plasma MCP-1 levels ~82% ↓*
	Macrophage infiltration ~83% ↓*
	Intima α-actin positive area ~77%↓*
	Smooth muscle cell content ~77%↓*
	Atherosclerotic lesions ↓*
	Intima-to-media ratio ~77% ↓*
Nasal instillation of antigens in OA-sensitized guinea pigs	Eosinophil count ~70% ↓*
[146]	
Mouse model of silicosis	Relieved impairment of pulmonary function
[12]	Alleviated abnormal right ventricular systolic pressure
	Normalized right ventricular hypertrophy index
	Attenuated lung fibrosis
	Reduced pulmonary artery remodeling
	Proinflammatory cytokines (TNF-α, IL-6, IL-1β, IL-18) ↓*
	Expression of NLRP3, caspase-1, & IL-1 β) ↓*

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.001$;

**** $p < 0.0001$

Table 3.

Effect of ramatroban (75 mg Bid) in clinical trials.

Clinical Trials [146]	Results
Double-blind controlled trial: 279 patients with allergic rhinitis	Final overall improvement 66.7% ↑*
Dose-response research study: 59 patients with moderate/severe perennial nasal allergy with nasal congestion	Final overall improvement 72.7% ↑* Improvement rate of nasal obstruction 90.9% ↑*
Randomized parallel dose-response study: 251 patients with severe perennial nasal allergy and moderate nasal congestion	Improvement of nasal congestion 69.8% ↑*

*$P < 0.05$