Ramatroban, an orally bioavailable immunomodulator and antithrombotic agent for treatment of COVID-19 disease: A Case Report

Ashutosh Agarwal, MBBS, MS
Charak Foundation of India, Indore, Madhya Pradesh, India
Kate Chander Chiang
Charak Foundation of India, Indore, Madhya Pradesh, India
Aditya Agarwal, MBBS, MS (adityaeyesite@gmail.com)
Charak Foundation of India, Indore, Madhya Pradesh, India

Case Report

Keywords: COVID-19, SARS-CoV-2, thromboxane A2, prostaglandin D2, lipid storm, ramatroban, DPr2, TPr, thromboinflammation, thrombosis, platelet activation, NETs, immunomodulator, antithrombotic

Posted Date: April 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-474882/v1

Abstract

SARS-CoV-2 pneumonia and COVID-19 disease are characterized by a maladaptive immune response and prothrombotic state. The Spike protein of SARS-CoV-2 virus unleashes a storm of lipid mediators with very high levels of thromboxane B_2 and prostaglandin D_2 in the bronchoalveolar lavage fluid. DPr2 receptors for prostaglandin D_2 and TPr receptors for thromboxane A_2 have been proposed as therapeutic targets in COVID-19 in order to decrease viral load and inhibit platelet dependent thrombosis, respectively. Ramatroban is an oral, dual receptor antagonist of the DPr2 and TPr receptors with established safety profile, having been used in Japan for the treatment of allergic rhinitis for over 20 years. We report two patients, an 88-year-old woman and a 31-year-old man, both with COVID-19 pneumonia who were successfully treated in ambulatory setting with oral ramatroban, leading to rapid improvement in oxygen saturation from about 80–82% to about 90–95% over 24–48 hours followed by gradual recovery from the disease. The mechanism of action of ramatroban and its efficacy reported here supports clinical trials on this drug as a potential treatment for COVID-19.

Background

During the current pandemic over 145 million patients have been infected with SARS-CoV-2 virus and more than 3 million have been deceased, as of April 2021. Despite vaccine rollout many countries continue to experience a surge in cases including India, Brazil, Chile, Canada and many European countries. COVID-19 disease is caused by SARS-CoV-2 virus which enters host cells through the ACE2 receptors. Severe SARS-CoV-2 infection is characterized by first, respiratory infection with the development of ARDS; and second, vascular disease manifesting as thrombotic microangiopathy, pulmonary thrombosis, pedal acro-ischemia (“COVID-toes”), arterial clots, strokes, cardiomyopathy, coronary and systemic vasculitis, bleeding, deep venous thrombosis, pulmonary embolism; and microvascular thrombosis in renal, cardiac and brain vasculature.[1–6]

The underlying pathobiology of SARS-CoV-2 infection comprises a trilogy of a maladaptive inflammatory response, immunosuppression and thromboinflammation. First, emerging evidence suggests that in patients with 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-13 and IL-5.[7–9] Second, IL-13 is known to upregulate monocyte-macrophage derived suppressor cells (MDSC), which play a role in immune suppression and lymphopenia.[10–12] Lymphopenia is one of the characteristic features of COVID-19 disease in adults, and a predictor of disease severity.[13] Third, endothelial cell injury by the SARS-CoV-2 virus and direct association of virus with platelets leads to platelet activation, release of platelet microvesicles, platelet-neutrophil and platelet-monocyte interaction and release of neutrophil extracellular traps (NETs).[5,14–16] This leads to inflammatory microvascular thrombi in the pulmonary, hepatic, renal and cardiac microvasculature and multi-organ failure.[1,5]

A proinflammatory lipid storm lies at the heart of the above trilogy. Archambault and colleagues 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.[17] Severe COVID-19 patients had marked increases in fatty acid levels as well as an accompanying inflammatory lipid storm with predominance of cyclooxygenase metabolites notably thromboxane B_2 > > PGE_2 > PGD_2.[17] PGD_2 action on DPr2 receptor mediates the maladaptive type 2 immune response.[18,19] Thromboxane A_2 action on thromboxane prostanoid receptors (TPr) mediates thromboinflammation.[11,12,20] DPr2 and TPr have been proposed as therapeutic targets in COVID-19, in order to decrease viral load and inhibit platelet-dependent thrombosis, respectively.[21] Ramatroban is the only dual receptor antagonist of the DPr2 and TPr receptors available for medicinal use. Ramatroban has an established safety profile having been used for over 20 years in Japan for the treatment of allergic rhinitis.[22,23] Here, we report the first use of ramatroban, a DPr2 and TPr antagonist to treat COVID-19 disease.

Case Reports

1st case

S.D., an 87-year old Indian lady (SD) experienced sudden onset of fever, cough, diarrhea, anorexia, profound weakness and slight shortness of breath, 10 days following a 2-hour flight from New Delhi to Indore, Madhya Pradesh, India. Patient had received the first dose of COVISHIELD vaccine 30 days prior to beginning of symptoms. Patient was examined by the first author. Patient was fully alert, oriented and able to make intelligent conversation but lay listlessly in bed unable to ambulate. Patient weighed 42 Kg and exhibited severe muscle wasting and marked kyphosis. Vital signs revealed temperature, 102^o Fahrenheit; heart rate, 100 per minute; blood pressure, 90/60 mm of Hg; and respiratory rate, 22 per minute. Mucosa were moist and mild pallor was present. There was no jugular venous distention or pedal edema. Chest examination revealed bilateral coarse rales especially prominent at both lung bases but no wheezes. Abdomen, cardiovascular and neurological examinations were unremarkable. Patient was not taking any medications.

Past medical history included hypertension for over 40 years; Hashimoto's thyroiditis with thyrotoxicosis for over 30 years treated with radioiodine therapy in 1999; severe osteoporosis with kyphosis; coronary artery disease leading to acute myocardial infarction and cardiac arrest in 2015 which required emergency coronary angioplasty and stent placement; chronic kidney disease with estimated glomerular filtration rate of about 20 mL/min.

Nasopharyngeal and oropharyngeal swabs were positive for SARS-CoV-2 infection by RNA PCR with cycle threshold (Ct range < 20 cycles). Pulse oximetry revealed oxygen saturation of about 85–88%. Patient was admitted on April 9, 2021 to Medanta Hospital, Indore, Madhya Pradesh, India. CT scan revealed moderate multifocal, patchy ground glass opacities, and consolidation. There was septal

thickening in the central and peripheral subpleural aspect of both lung parenchyma. Serial laboratory examinations during the course of the illness are listed in Table 1.

During the hospital stay the patient was treated with intravenous remdesivir, antibiotics and methylprednisolone. In addition, prophylactic low-molecular weight heparin was administered subcutaneously in a dose of 40 mg twice a day. Patient required high-flow nasal oxygen during the hospital stay. Patient continued to have fever, cough, shortness of breath, diarrhea and profound weakness during the hospital stay. Oxygen saturation on room air ranged between 82–86%. After a hospital stay of 5 days the patient was discharged upon her request on April 14, 2021. Discharge medications included oral oseltamivir, doxycycline, vitamin C, aspirin 75 mg once a day, 5 mg prednisolone, vitamin D_3, and nebulization with budesonide and salbutamol twice daily. Supportive management with betadine gargles, steam inhalation and breathing exercises was continued.

On April 15, the day after discharge from the hospital, the patient had fever with a temperature of 101^{o} Fahrenheit. Pulse oximetry revealed an oxygen saturation of 82–84%. Patient was profoundly weak and unable to get out of bed without assistance. At this time all drugs including low-dose aspirin were discontinued and the patient was started on ramatroban in a dose of 75 mg tablet, one-half tablet (37.5 mg) twice daily. Within 36 hours, after having received three doses of ramatroban, patient's oxygen saturation improved to 90% on room air and there was progressive improvement in her general condition. The dose of ramatroban was increased to 37.5 mg in the morning and 75 mg at bedtime. Patient had complete resolution of cough and diarrhea over a period of next 3 days, and started ambulating independently without assistance. Patient had recovered almost completely by April 22, 2021.

Table 1
Serial laboratory values

Analytes	Before admission April 8, 2021	During hospital stay April 13, 2021	After discharge April 16, 2021	Reference Value
Hemoglobin (g/dl)	11.7	12.1	12.0	13.0–17.0
Platelet count (per mm^3)	214,000	285,000	402,000	150,000-410,000
RBC count (x 10^2/L)	3.77	3.93	4.01	4.5–5.5
WBC count (per mm^3)	5040	12100	9010	4000–10000
Neutrophils (%)	77	80	86	38–70
Lymphocytes (%)	18	11	07	21–49
NLR (Neutrophil:Lymphocyte Ratio)	4.3	7.3	12.3	1.1–3.5
Serum CRP (mg/L)	7.86	35.9	15.3	0–5.0
D-dimer (ng FEU/mL)	600	650	659	< 500

2nd case

A 31-year old businessman at New Delhi experienced sudden onset of fever, cough and dyspnea. The nasopharyngeal and oropharyngeal swabs were positive for SARS-CoV-2 infection by RNA PCR. Pulse oximetry revealed an oxygen saturation of 80–82%. Patient was started on ramatroban 75 mg twice daily. Within 36 hours the patient experienced remarkable improvement in symptoms and oxygen saturation improved to 90–95%. Patient did not require admission to the hospital.

These case reports are retrospective descriptions of two patients, their laboratory profile and clinical course. Consent or waiver of such consent was deemed unnecessary and unwarranted considering the patients have not been identified in the case report.

Discussion

The first case presented above is consistent with classical clinical manifestations of moderate to severe COVID-19 disease including hypoxia, lymphopenia, neutrophilia, and increased plasma levels of C-reactive protein and D-dimers. This case represents the trilogy of maladaptive inflammatory response, immunosuppression and thromboinflammation in COVID-19, which is fueled by a proinflammatory lipid storm with massive generation of thromboxane A_2 and PGD_2.[17] It has been proposed that early

administration of well-tolerated antagonist of PGD_2/DPr2 and thromboxane A_2/TPr signaling may limit progression to severe COVID-19.[21]

Ramatroban is the only approved dual receptor antagonist of the DPr2 and TPr receptors. Ramatroban (Baynas®, Bayer Yakuhin Ltd., Japan) has been safely used for the treatment of allergic rhinitis in Japan since 2000.[23] The usual adult oral dose of 75 mg twice daily achieves an average plasma concentration of about 0.1 mg/L or 240 nM which is sufficient to inhibit platelet activation since the IC_{50} for human platelet aggregation is only about 30 nM.[23] Ramatroban exhibits surmountable binding to thromboxane A_2 receptors.[24] Ramatroban is 100 times more potent than aspirin in inhibiting platelet aggregation and P-selectin expression.[23,25] With a plasma half-life of about 2 hours the antiplatelet action of ramatroban is reversible.[23] This is of advantage in the event of bleeding complications which have been reported in > 5% of critically ill COVID-19 patients.[26] Ramatroban is primarily metabolized in the liver through the acyl glucuronic acid conjugate metabolic pathway, is excreted mainly in the bile; and therefore can be safely administered in the presence of acute kidney injury or chronic kidney disease.[27] The blood concentration of ramatroban in the elderly (≥ 65 years of age) is estimated to be higher than in the non-elderly population, and hence the starting dose in the elderly is 50 mg twice a day.[27]

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.[23] In a rat model of endotoxic shock, ramatroban prevented hypotension, reduced plasma TNF levels by over 90%, and markedly reduced myeloperoxidase levels in lungs, ileum and heart, suggesting end-organ protection by mitigating thromboxane A_2 mediated platelet-polymorphonuclear leukocyte activation, and improved survival by 45%.[28] In rats with splanchnic artery ischemia-reperfusion injury, while plasma levels of thromboxane B_2 were increased about 7-fold, ramatroban prevented hypotension, improved survival, restored phagocytic function of peritoneal macrophages partially, inhibited plasma myocardial depressant factor activity about 50%, and inhibited tissue infiltration by neutrophils as measured by decline in ilium myeloperoxidase activity > 50% and lung myeloperoxidase activity > 80%.[29] Notably, myeloperoxidase is significantly increased in COVID-19, is abundant in NETs and regulates NET formation via synergy with neutrophil elastase.[30,31] Therefore, ramatroban is remarkably effective in both endotoxin and ischemia reperfusion injury induced shock states which share common pathogenetic mechanisms with severe COVID-19.

These are the first reported cases of COVID-19 treated with ramatroban. The rapid recovery in both cases including the frail, 88 years old patient with chronic kidney disease (eGFR of about 20 mL/minute) and coronary artery disease, despite failure of conventional treatment regimen with remdesivir and steroids, appears to coincide with institution of ramatroban. Such a clinical response is consistent with the potent immunomodulatory and antithrombotic actions of ramatroban observed in preclinical studies. Therefore, in patients with COVID-19, ramatroban appears to have the potential to prevent microvascular thrombosis,[28,32] limit viral load by promoting interferon lambda (IFN-λ) response,[19] and reduce disease

severity by mitigating the maladaptive shift from Th1 to Th2 immune responses.[11] The clinical response to ramatroban in the two cases reported, its established safety profile and its oral bioavailability support further clinical trials of this drug as a potential treatment for COVID-19.

Abbreviations

Tx, thromboxane; PG, prostaglandin; COX, cyclooxygenase; DPr2, D prostanoid receptor 2; COX, cyclooxygenase; NET, neutrophil extracellular trap; BALF, Bronchoalveolar lavage fluid; COVID-19, coronavirus disease 2019; IL, interleukin; MDSC, monocyte-macrophage derived suppressor cells; RSV, respiratory syncytial virus; IFN, interferon; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Declarations

Consent: The patient consented to participate and wishes to publish their clinical data.

Conflict of Interest:
There was no conflict of interest of the authors responsible for the management of the patient including the use of ramatroban.

References

1. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *New England Journal of Medicine.* 2020.
2. Goyal P, Choi JJ, Pinheiro LC, et al. Clinical Characteristics of Covid-19 in New York City. *N Engl J Med.* 2020;382(24):2372–2374.
3. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine.* 2020;382(18):1708–1720.
4. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, et al. Platelet activation and platelet-monocyte aggregates formation trigger tissue factor expression in severe COVID-19 patients. *Blood.* 2020.
5. Nicolai L, Leunig A, Brambs S, et al. Immunothrombotic Dysregulation in COVID-19 Pneumonia is Associated with Respiratory Failure and Coagulopathy. *Circulation.* 2020.
6. Song W-C, Fitzgerald GA. COVID-19, microangiopathy, hemostatic activation, and complement. *Journal of Clinical Investigation.* 2020.
7. Yang L, Liu S, Liu J, et al. COVID-19: immunopathogenesis and Immunotherapeutics. *Signal Transduction and Targeted Therapy.* 2020;5(1).
8. Lucas C, Wong P, Klein J, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature.* 2020;584(7821):463–469.
9. Perlman S. COVID-19 poses a riddle for the immune system. *Nature.* 2020;584(7821):345–346.

10. Arima M, Fukuda T. Prostaglandin D2and TH2 Inflammation in the Pathogenesis of Bronchial Asthma. *The Korean Journal of Internal Medicine.* 2011;26(1):8.
11. Xue L, Gyles SL, Wettey FR, et al. Prostaglandin D2 Causes Preferential Induction of Proinflammatory Th2 Cytokine Production through an Action on Chemoattractant Receptor-Like Molecule Expressed on Th2 Cells. *The Journal of Immunology.* 2005;175(10):6531–6536.
12. Trabanelli S, Chevalier MF, Martinez-Usatorre A, et al. Tumour-derived PGD2 and NKp30-B7H6 engagement drives an immunosuppressive ILC2-MDSC axis. *Nat Commun.* 2017;8(1):593.
13. Liu J, Li H, Luo M, et al. Lymphopenia predicted illness severity and recovery in patients with COVID-19: A single-center, retrospective study. *PLOS ONE.* 2020;15(11):e0241659.
14. Rapkiewicz AV, Mai X, Carsons SE, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: A case series. *EClinicalMedicine.* 2020;24:100434.
15. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. *Journal of Experimental Medicine.* 2020;217(6).
16. Zaid Y, Puhm F, Allaeys I, et al. Platelets Can Associate With SARS-CoV-2 RNA and Are Hyperactivated in COVID-19. *Circulation Research.* 2020;127(11):1404–1418.
17. Archambault A-S, Zaid Y, Rakotoarivelo V, et al. Lipid storm within the lungs of severe COVID-19 patients: Extensive levels of cyclooxygenase and lipoxygenase-derived inflammatory metabolites. *medRxiv.* 2020:2020.2012.2004.20242115.
18. Domingo C, Palomares O, Sandham DA, Erpenbeck VJ, Altman P. The prostaglandin D2 receptor 2 pathway in asthma: a key player in airway inflammation. *Respiratory Research.* 2018;19(1).
19. Werder RB, Lynch JP, Simpson JC, et al. PGD2/DP2 receptor activation promotes severe viral bronchiolitis by suppressing IFN-lambda production. *Sci Transl Med.* 2018;10(440).
20. Rucker D, Dhamoon AS. Physiology, Thromboxane A2. In: *StatPearls.* Treasure Island (FL)2020.
21. Theken KN, Fitzgerald GA. Bioactive lipids in antiviral immunity. *Science.* 2021;371(6526):237–238.
22. Uller L, Mathiesen JM, Alenmyr L, et al. Antagonism of the prostaglandin D2 receptor CRTH2 attenuates asthma pathology in mouse eosinophilic airway inflammation. *Respiratory Research.* 2007;8(1).
23. Ishizuka T, Matsui T, Okamoto Y, Ohta A, Shichijo M. Ramatroban (BAY u 3405): a novel dual antagonist of TXA2 receptor and CRTh2, a newly identified prostaglandin D2 receptor. *Cardiovasc Drug Rev.* 2004;22(2):71–90.
24. Mathiesen JM, Christopoulos A, Ulven T, et al. On the Mechanism of Interaction of Potent Surmountable and Insurmountable Antagonists with the Prostaglandin D2 Receptor CRTH2. *Molecular Pharmacology.* 2006;69(4):1441–1453.
25. Kariyazono H, Nakamura K, Arima J, et al. Evaluation of anti-platelet aggregatory effects of aspirin, cilostazol and ramatroban on platelet-rich plasma and whole blood. *Blood Coagul Fibrinolysis.* 2004;15(2):157–167.

26. Al-Samkari H, Karp Leaf RS, Dzik WH, et al. COVID and Coagulation: Bleeding and Thrombotic Manifestations of SARS-CoV2 Infection. *Blood.* 2020.
27. Nippon Shinyaku Co. Ltd. Prostaglandin D2 and Thromboxane A2 receptor antagonists; Medicine for allergic rhinitis; Baynas tablets package insert. https://www.nippon-shinyaku.co.jp/assets/files/pdfs/medicine/product/ha/baynas/interview_baynas_t.pdf. Published 2009. Accessed April 27, 2021.
28. Altavilla D, Canale P, Squadrito F, et al. Protective effects of BAY U 3405, a thromboxane A2 receptor antagonist, in endotoxin shock. *Pharmacol Res.* 1994;30(2):137–151.
29. Canale P, Squadrito F, Altavilla D, et al. Beneficial effects of BAY u3405, a novel thromboxane A2 receptor antagonist, in splanchnic artery occlusion shock. *Pharmacology.* 1994;49(6):376–385.
30. Skendros P, Mitsios A, Chrysanthopoulou A, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *Journal of Clinical Investigation*. 2020.
31. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol.* 2010;191(3):677–691.
32. Squadrito F, Ioculano M, Altavilla D, et al. Reduction of myocardial leukocyte accumulation and myocardial infarct size following administration of BAY u3405, a thromboxane A2 receptor antagonist, in myocardial ischaemia-reperfusion injury. *Agents and Actions.* 1993;39(3–4):143–149.