Targeting lymphocyte Kv1.3-channels to suppress cytokine storm in severe COVID-19: Can it be a novel therapeutic strategy?

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SUMMARY   In the midst of a pandemic, finding effective treatments for coronavirus disease 2019 (COVID-19) is the urgent issue. In "chronic inflammatory diseases", the overexpression of delayed rectifier K^+-channels (Kv1.3) in leukocytes is responsible for the overactivation of cellular immunity and the subsequent cytokine storm. In our previous basic studies, drugs including chloroquine and azithromycin strongly suppressed the channel activity and pro-inflammatory cytokine production from lymphocytes. These findings suggest a novel pharmacological mechanism by which chloroquine, with or without azithromycin, is effective for severe cases of COVID-19, in which the overactivation of cellular immunity and the subsequent cytokine storm are responsible for the pathogenesis.

*Keywords*   COVID-19, cytokine storm, lymphocyte, Kv1.3-channels, chloroquine

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Most patients are either asymptomatic or develop only mild to moderate symptoms, such as fever, dry cough and shortness of breath, which often improve spontaneously with supportive treatment alone. However, some patients develop fatal pneumonia with acute respiratory distress syndrome (ARDS) or are occasionally complicated by multiple organ failure due to generalized thrombotic microangiopathy as the result of systemic vasculitis (*1*). Concerning the mechanisms, the over-activation of leukocytes and the uncontrolled release of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1, IL-2, IL-10, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, are thought to be responsible (*2,3*). This phenomenon is termed "cytokine storm" characterized by an excessive response of the immune system.

"Chronic inflammatory diseases" is a disease category in which over-stimulated cellular immunity is responsible for the pathogenesis (*4*). Besides infectious diseases and autoimmune disorders, studies indicate that common diseases, such as metabolic disorders and cancer, are also included in this disease category. Recently, we have demonstrated in animal studies that that the cytokine storm can occur in advanced-stage kidney diseases, such as end-stage renal disease and interstitial nephritis (*5,6*). In these animal models, leukocytes including T-lymphocytes and macrophages were markedly increased within the kidneys and the

cytokine levels, such as IL-2 and TNF-α, were actually elevated. In these leukocytes, since delayed rectifier K^+-channels (Kv1.3) were over-expressed, and since pharmacological blockade of the channels actually ameliorated the disease progression, they were thought to be the primary trigger of the overactivation of cellular immunity and the subsequent cytokine storm.

In the midst of a pandemic, finding effective treatments for COVID-19 is an urgent issue. Despite the limited evidence, some clinical studies have suggested the efficacy of chloroquine with or without azithromycin for COVID-19 patients with severe respiratory failure (*7-9*). In addition to the direct effect of chloroquine on reducing the viral replication (*10*), in vitro studies demonstrated its inhibitory effect on the production of cytokines from leukocytes (*7,11*). Regarding the mechanisms of such anti-inflammatory properties of chloroquine, recent reports suggest the involvement of glycogen synthase kinase-3β (GSK3β) or Toll-like receptors (TLRs) (*12,13*). In our previous patch-clamp studies using murine thymocytes, both chloroquine and azithromycin strongly suppressed the activity of lymphocyte Kv1.3-channels and thus reduced the production of inflammatory cytokines (*14,15*). These findings provide an additional pharmacological mechanism by which chloroquine with or without azithromycin was effective for severe cases of COVID-19, where the overactivation of cellular immunity and the subsequent cytokine storm were responsible for their pathogenesis (Figure 1).

Additionally, in our series of patch-clamp studies
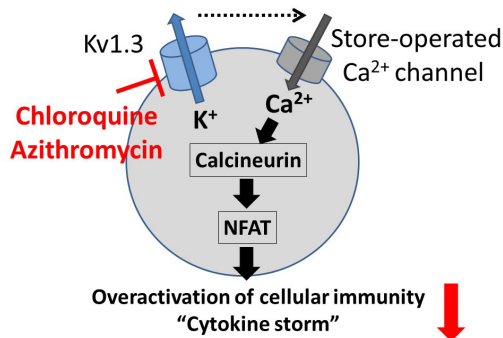
Figure 1. Roles of Kv1.3-channels in the activation pathway of T lymphocytes and as the targets of chloroquine and azithromycin. Kv1.3-channels promote calcium influx and trigger the proliferation and activation of lymphocytes (*4*). The increased cytosolic calcium concentration stimulates the phosphatase calcineurin, which dephosphorylates the nuclear factor of activated T cells (NFAT), causing its accumulation in the nucleus and binding to the promoter region of cytokine-encoding genes. Both chloroquine and azithromycin inhibit Kv1.3-channels (*14,15*).

so far, we also revealed the inhibitory properties of nonsteroidal anti-inflammatory drugs, anti-hypertensive drugs, anti-cholesterol drugs and anti-allergic drugs on lymphocytes Kv1.3-channels (*16-18*). Considering such pharmacological properties, these commonly used drugs may also be beneficial in the treatment of COVID-19, since the channel blockade reduces the cytokine production and thus suppresses the cytokine storm.

Acknowledgements

References

1. Leisman DE, Deutschman CS, Legrand M. Facing COVID-19 in the ICU: vascular dysfunction, thrombosis, and dysregulated inflammation. Intensive Care Med. 2020; 46:1105-1108.
2. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev. 2020; 53:25-32.
3. Hirano T, Murakami M. COVID-19: A New Virus, but a Familiar Receptor and Cytokine Release Syndrome. Immunity. 2020; 52:731-733.
4. Kazama I. Physiological significance of delayed rectifier K^+ channels (Kv1.3) expressed in T lymphocytes and their pathological significance in chronic kidney disease. J Physiol Sci. 2015; 65:25-35.
5. Kazama I, Baba A, Matsubara M, Endo Y, Toyama H, Ejima Y. Benidipine suppresses in situ proliferation of leukocytes and slows the progression of renal fibrosis in rat kidneys with advanced chronic renal failure. Nephron Exp Nephrol. 2014; 128:67-79.
6. Abe N, Toyama H, Saito K, Ejima Y, Yamauchi M, Mushiake H, Kazama I. Delayed rectifier K^+-channel is a novel therapeutic target for interstitial renal fibrosis in rats with unilateral ureteral obstruction. Biomed Res Int. 2019; 2019:7567638.
7. Shukla AM, Archibald LK, Wagle Shukla A, Mehta HJ, Cherabuddi K. Chloroquine and hydroxychloroquine in the context of COVID-19. Drugs Context. 2020; 9:2020-4-5.
8. Dallan C, Romano F, Siebert J, Politi S, Lacroix L, Sahyoun C. Septic shock presentation in adolescents with COVID-19. Lancet Child Adolesc Health. 2020; 4:e21-e23.
9. Borba MGS, Val FFA, Sampaio VS, *et al.* Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: A randomized clinical trial. JAMA Netw Open. 2020; 3:e208857.
10. Cortegiani A, Ippolito M, Ingoglia G, Einav S. Chloroquine for COVID-19: rationale, facts, hopes. Crit Care. 2020; 24:210.
11. van den Borne BE, Dijkmans BA, de Rooij HH, le Cessie S, Verweij CL. Chloroquine and hydroxychloroquine equally affect tumor necrosis factor-alpha, interleukin 6, and interferon-gamma production by peripheral blood mononuclear cells. J Rheumatol. 1997; 24:55-60.
12. Embi MN, Ganesan N, Sidek HM. Is GSK3beta a molecular target of chloroquine treatment against COVID-19? Drug Discov Ther. 2020; 14:107-108.
13. Sahraei Z, Shabani M, Shokouhi S, Saffaei A. Aminoquinolines against coronavirus disease 2019 (COVID-19): chloroquine or hydroxychloroquine. Int J Antimicrob Agents. 2020; 55:105945.
14. Kazama I, Maruyama Y, Murata Y, Sano M. Voltage-dependent biphasic effects of chloroquine on delayed rectifier K^+-channel currents in murine thymocytes. J Physiol Sci. 2012; 62:267-274.
15. Kazama I, Maruyama Y. Differential effects of clarithromycin and azithromycin on delayed rectifier K^+-channel currents in murine thymocytes. Pharm Biol. 2013; 51:760-765.
16. Kazama I, Tamada T, Tachi M. Usefulness of targeting lymphocyte Kv1.3-channels in the treatment of respiratory diseases. Inflamm Res. 2015; 64:753-765.
17. Baba A, Tachi M, Maruyama Y, Kazama I. Suppressive effects of diltiazem and verapamil on delayed rectifier K+-channel currents in murine thymocytes. Pharmacol Rep. 2015; 67:959-964.
18. Saito K, Abe N, Toyama H, Ejima Y, Yamauchi M, Mushiake H, Kazama I. Second-generation histamine H1 receptor antagonists suppress delayed rectifier K^+-channel currents in murine thymocytes. Biomed Res Int. 2019; 2019:6261951.

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