Pioglitazone treatment for the COVID-19-associated cytokine storm

Adnan EROL, MD.
Professor of internal medicine
EPDH for the disorders of energy metabolism
Silivri-Istanbul, Turkey

email: eroladnan@protonmail.com

Accumulating evidence suggests that a subgroup of patients with severe COVID-19 might have a cytokine storm syndrome, suggesting that mortality may be due to virally driven hyper-inflammation [1]. Patients who develop myocardial injury with COVID-19 have clinical evidence of a higher incidence of acute respiratory distress syndrome and more frequent need for assisted ventilation than those without myocardial injury, and the patients who are more prone to have myocardial injury are older patients with preexisting cardiovascular complications and diabetes [2]. A 12-year follow-up survey of 25 patients who recovered from SARS-CoV infection found that 68% had hyperlipidemia, 44% had cardiovascular system abnormalities, and 60% had glucose metabolism disorders. A meta-analysis showed that SARS-CoV infection was more likely to occur in patients with underlying cardiovascular disease. In patients with SARS-CoV infection and severe symptoms, 50% had hypertension and diabetes and up to 30% had heart disease. Given that SARS-CoV-2 has a similar structure to SARS-CoV, patients with cardiovascular disease are more likely to develop severe symptoms if infected with SARS-CoV-2 [3]. Since the common denominator of these diseases is accelerated atherosclerotic process, it is required to closely examine the relationship between atherosclerotic pathology and COVID-19.

Atherosclerotic process and macrophage activity

In COVID-19, host disease is likely a combination of direct viral damage and consequences of an aberrant immune response promoted by inflammatory monocyte-macrophages [4]. Host macrophages can be reprogrammed into opposing primed or tolerant states depending upon the nature and quantities of external stimulants. Several antigenic stimulant priming, including oxidized low density lipoprotein (oxLDL), may alert the host to the presence of infection. Subsequently, exacerbated responses in low dose oxLDL-primed individuals during severe infection may contribute to higher mortality [5].

Many host-derived molecules, ranging from lipids to proteins, exert cellular responses via TLR4. The common feature of host derived TLR4 ligands is they are modified, but not native, host molecules. One of the host-derived TLR4 ligands is minimally oxidized LDL (moxLDL), a lipoprotein composed of hundreds of lipid and sterol molecules, but not native LDL. In addition to TLR4, moxLDL likely engages several other receptors, leading to their clustering with TLR4 [6].

Atherosclerosis is a disease associated with increased titers of immune complexes, containing antibodies directed to moxLDLs. In fact, up to 90% of circulating moxLDL can be found in oxLDL immune complexes (oxLICs). FcγRs (receptor for the Fc portion of IgG) are the canonical receptors for IgG-containing immune complexes; however, moxLDLs activate the inflammasome via TLR4 and scavenger receptors. Therefore, oxLICs prime the inflammasome in macrophages in a collaboration of FcγR, TLR4, and CD36 [7]. Remarkably, in SARS-CoV-infected lungs, local activation of the oxidative stress reasons in the marked production of oxidized phospholipids, which are the primary active species of ox-LDL. Thus, the acute onset of pro-inflammatory cytokine production in primed-macrophages by oxLICs and severe lung injury caused by different pathogens critically depends on activation of the oxidative stress machinery that couples to innate immunity [8].

Macrophages are present in virtually all tissues, where they integrate a large number of inputs to coordinate developmental, metabolic and immune functions, thus critically contributing to maintain homeostasis [9]. oxLIC-mediated inflammasome priming in macrophages is CARD9-dependent. [7]. CARD9 is critical adaptor protein that is operated downstream of pattern recognition receptors. CARD9 signalosome, which is a tripartite protein complex formed from CARD9, BCL10, and MALT1 (CBM complex), leads to the

activation of NF- κ B and mitogen-activated protein kinase (MAPK) signaling, resulting upregulation of several cytokines and chemokines [10]. BCL10 consists of an N-terminal CARD domain, which typically mediates homotypic protein interactions, and a C-terminal serine-threonine rich region that contain several phosphorylation sites to regulate BCL10 function. Mind bomb homologue 2 (MIB2) is an E3 ligase and essential component of the activated BCL10 signaling complex. MIB2 has two MIB/HERC2 domains, a zinc finger domain, several ankyrin repeat domains, and two C-terminal RING finger domains. BCL10-MIB2 association is chiefly mediated via an unconventional, heterotypic CARD-MIB/HERC2 domains. The activated CBM complex recruits MIB2 into complex. Subsequently, activation of MIB2 leads to the K63-linked auto-ubiquitination of itself, which, in turn, stimulates the recruitment of TAB proteins along with TAK1 and IKK γ (NEMO) [11]. TAK1 is an ubiquitin-dependent master kinase known to phosphorylate and activate IKK β and JNK [12]. MALT1, another component of CBM complex, promotes the recruitment of IKK complex and acts as a protease, cleaving the negative regulatory molecule A20. Thus, MIB2 interaction with CBM complex ultimately results in NF- κ B activation [11].

MIB2 also interacts with cylindromatosis (CYLD) deubiquitinase. The ankyrin repeat region of MIB2 interacts with third CAP-glycine domain of CYLD. MIB2-dependent ubiquitination of CYLD induces its degradation by the proteasome. CYLD deubiquitinates two types of polyubiquitination, referred as K63-linked polyubiquitin and M1-linked linear ubiquitin chains [13]. CYLD physically interacts with TAK1 and NEMO, inhibits their ubiquitination and activity. Thus, CYLD inhibits the activation of NF- κ B and MAPKs stimulated by innate immune receptors [12]. Altogether, oxLIC-CBM signaling pathway-stimulated MIB2 may lead to the unregulated NF- κ B and MAPKs activation by the inhibition of major deubiquitinase, CYLD (Figure).

SARS-CoV-mediated macrophage infection

It is now agreed that SARS-CoV can infect not only the respiratory tract, but can also other organ systems, including hematopoietic cells. However, immune cells, including macrophages, do not express the SARS-CoV receptor angiotensin-converting enzyme 2 (ACE2). Remarkably, anti-Spike antibodies against SARS-CoV Spike protein trimers potentiate the infection of both monocytic and lymphoid immune cells. Thus, human macrophages can be infected by SARS-CoV as a result of antibody (IgG)-dependent enhancement (ADE) process. This infection route requires signaling pathways activated downstream of binding to Fc γ R2 receptors [14].

SARS-CoVs are large, enveloped, positive RNA viruses. The cytoplasmic virus receptor RIG-I directly recognizes and binds viral 5'-PPP-RNA via its C-terminal repressor domain (RD). Thus, viral infection-induced conformational change exposes caspase recruitment domains (CARDs) of RIG-I. In open conformation, CARDs capture and stabilize K63-linked polyubiquitin chains synthesized by the E3 ubiquitin ligase, TRIM25. Subsequently, RD releases auto-repression of RIG-I and facilitates oligomerization, leading to antiviral signaling cascade by interacting with downstream partner mitochondrial antiviral-signaling (MAVS) [15]. TRIM25 is also capable of promoting K-48-linked ubiquitination and degradation of MAVS. Surprisingly, MAVS degradation is required for the downstream signaling for type I IFN production [16]. Once activated, MAVS develop a functional prion-like structure at mitochondria, leading to the phosphorylation of IRF3 and subsequent transcription of type I IFNs [17].

Viruses have evolved the capacity to evade host immune recognition and to suppress to host IFN system. SARS-CoV and MERS-CoV N proteins bind to the SPRY domain of TRIM25, therefore suppress TRIM25-mediated RIG-I and MAVS modulations, and

subsequently type I IFN production. This is important to provide comprehensive insight into the mechanism of SARS-CoV-induced innate immune suppression [18]. SARS-CoV-induced a delayed type I interferon response promotes high initial virus titers and further aberrant inflammatory monocyte-macrophages recruitment. A robust elevation of IL-1 β was seen during early infection of SARS-CoV. IL-1 β is a key proinflammatory cytokine generated from pro-IL-1 β through proteolytic cleavage by caspase 1, which is activated by multiprotein inflammasome complexes [4].

RIG-I helicase, in addition to synthesis of type I interferons, also activates the pro-inflammatory NF- κ B pathway for the production of cytokines, such as IL-1 β and IL-6. The production of IL-1 β requires, in addition to NF- κ B-dependent new synthesis pro-IL-1 β , a second signal that triggers caspase-1 activation for bioactive mature IL-1 β . RIG-I serves as a dual sensor that can trigger both NF- κ B-dependent production of pro-IL-1 β and inflammasome activation in response to certain RNA viruses. RIG-I engages the CARD9-BCL10 module for NF- κ B activation and triggers ASC for inflammasome activation in an NLRP3-independent manner. Although MAVS signaling is required for RIG-I-mediated production of Type I interferons and IL-1 β , MAVS engagement is not involved in RIG-I-mediated activation of caspase-1. TRIM25-deficient cells showed defects in type I interferons and interleukins after viral infection; however, caspase-1 was activated normally in these cells. Altogether, while SARS-CoV-infected and OxLICs-primed macrophages cannot produce protective type I interferons, they readily potentiates existing inflammation through ASC-mediated caspase-1 activation [19] (Figure).

Thiazolidinediones against cytokine storm

A coordinated cytokine response is essential for creating an appropriate host immune response and for its resolution after the inflammatory trigger disappears. Due to CYLD inhibition in oxLICs-primed macrophages, pro-inflammatory cytokines are left unconfined. In fact, it is believed that a dysregulated cytokine response can lead to a hyper-inflammatory condition called “cytokine storm”, which may lead to further organ injury. Considering the CARD9 adaptor protein is in the very heart of the macrophage priming process, inhibition of its activity may forestall the SARS-CoV2-mediated potentiation of the hyper-inflammatory macrophage response.

SARS-CoV-2 infection is triggered by binding of the spike protein of the virus (SARS-S) to ACE2, which is highly expressed in lungs and heart. Therefore, although the symptoms of COVID-19 are mostly respiratory, some patients suffer severe cardiovascular damage. Importantly, ACE2 levels can be increased by the use of renin-angiotensin system (RAS) inhibitors [3]. RAS is one of the central players in respiratory diseases, such as acute respiratory distress syndrome (ARDS) and acute lung injury during sepsis. ACE2 acts as an anti-inflammatory protein by counteracting the actions of Angiotensin II. Thus, ACE2, a homologue of ACE1, has a protective function in lung injury due to the degradation of angiotensin II [20]. However, ACE2 functions are suppressed by SARS-CoV-2 spike protein (SARS-S), which may promote the activity of SARS-CoV2 [21]. ACE2 overexpression inhibits both inflammation and TLR4 signaling pathway [22]. Thus, SARS-CoV-2 association with ACE2 and blocking its function may release the TLR4-mediated lethal pulmonary complications

Accumulating evidence indicate that peroxisome proliferator-activated receptor-gamma (PPAR- γ) is expressed in different immune cells, such as macrophages, dendritic cells, and T cells. PPAR- γ , belongs to a nuclear hormone receptor family, is a transcription factor that has been shown to play an important role in inflammation and immunity. It is accepted that PPAR- γ is a negative regulator of macrophage activation and T cell responses

[23]. PPAR- γ ligands can decrease the secretion of TNF- α , IL-1 β , and IL-6 in monocytes and macrophages [24]. It has been shown that PPAR γ activation by its high-affinity synthetic ligand, troglitazone, impairs nuclear localization of NF- κ B through inhibition of CARD9 expression [25]. Troglitazone has also a unique inhibitory action on macrophage differentiation, which is mediated by suppressing NF- κ B activation independently of PPAR γ [26]. However, troglitazone was withdrawn from the market in 2000 due to concerns over increasing reports of serious hepatic adverse events.

Pioglitazone is another available thiazolidinedione that also inhibits the activation of NF- κ B and MAPK pathways by reducing the expression of CARD9 [27]. In addition, pioglitazone attenuates the dendritic cell trafficking, antigen uptake and dendritic cell-T cell interactions at the initiation of immune response [23]. Pioglitazone treatment also increases serum adiponectin levels and decreases mortality from sepsis and lung injury by reducing inflammatory cytokine production in omental tissue [28]. Furthermore, pioglitazone upregulates ACE2 expression, supporting the control of ongoing inflammation [29]. Finally, the anti-inflammatory and insulin-sensitizing activities of pioglitazone are two distinctly separable effects. A sub-therapeutic dose of pioglitazone produces anti-inflammatory effects by suppressing TNF α and IL-6 without altering metabolic parameters [30]. Therefore, pioglitazone, in 15mg/day dose, seems to be preferred as a treatment option to produce anti-inflammatory effects for COVID-19 patients.

References

1. Mehta P, McAuley DF, Brown, M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; 395 (10229): 1033-1034, DOI: 10.1016/S0140-6736(20)30628-0.
2. Guo T, Fan Y, Chen M, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 2020;e201017. doi:10.1001/jamacardio.2020.1017.
3. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* 2020;10.1038/s41569-020-0360-5. doi:10.1038/s41569-020-0360-5.
4. Yuen KS, Ye ZW, Fung SY, Chan CP, Jin DY. SARS-CoV-2 and COVID-19: The most important research questions. *Cell Biosci.* 2020;10:40. doi:10.1186/s13578-020-00404-4.
5. Deng H, Maitra U, Morris M, Li L. Molecular mechanism responsible for the priming of macrophage activation. *J Biol Chem.* 2013; 288: 3897–3906. doi:10.1074/jbc.M112.424390.
6. Miller YI, Choi SH, Wiesner P, Bae YS. The SYK side of TLR4: signalling mechanisms in response to LPS and minimally oxidized LDL. *Br J Pharmacol.* 2012; 167: 990–999. doi:10.1111/j.1476-5381.2012.02097.x.
7. Rhoads JP, Lukens JR, Wilhelm AJ, et al. Oxidized Low-Density Lipoprotein immune complex priming of the Nlrp3 Inflammasome involves TLR and Fc γ R cooperation and is dependent on CARD9. *J Immunol.* 2017; 198: 2105–2114. doi:10.4049/jimmunol.1601563.
8. Imai Y, Kuba K, Neely GG, et al. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell.* 2008; 133: 235-49.
9. Glass CK, Natoli G. Molecular control of activation and priming in macrophages. *Nat Immunol.* 2016; 17: 26–33. doi:10.1038/ni.3306.

10. Zhong X, Chen B, Yang L, Yang Z. Molecular and physiological roles of the adaptor protein CARD9 in immunity. *Cell Death Dis.* 2018; 9: 52. doi:10.1038/s41419-017-0084-6.
11. Stempin CC, Chi L, Giraldo-Vela JP, High AA, Häcker H, Redecke V. The E3 ubiquitin ligase mind bomb-2 (MIB2) protein controls B-cell CLL/lymphoma 10 (BCL10)-dependent NF- κ B activation. *J Biol Chem.* 2011; 286: 37147–37157. doi:10.1074/jbc.M111.263384.
12. Reiley WW, Jin W, Lee AJ, et al. Deubiquitinating enzyme CYLD negatively regulates the ubiquitin-dependent kinase Tak1 and prevents abnormal T cell responses. *J Exp Med.* 2007; 204: 1475–1485. doi:10.1084/jem.20062694.
13. Uematsu A, Kido K, Takahashi H, et al. The E3 ubiquitin ligase MIB2 enhances inflammation by degrading the deubiquitinating enzyme CYLD. *J Biol Chem.* 2019; 294: 14135–14148. doi:10.1074/jbc.RA119.010119.
14. Yip MS, Leung NH, Cheung CY, et al. Antibody-dependent infection of human macrophages by severe acute respiratory syndrome coronavirus. *Virol J.* 2014; 11: 82. doi:10.1186/1743-422X-11-82.
15. Gack MU, Shin YC, Joo CH, et al. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature.* 2007; 446: 916-920.
16. Castanier C, Zemirli N, Portier A, et al. MAVS ubiquitination by the E3 ligase TRIM25 and degradation by the proteasome is involved in type I interferon production after activation of the antiviral RIG-I-like receptors. *BMC Biol.* 2012; 10: 44.
17. Hou F, Sun L, Zheng H, Skaug B, Jiang QX, Chen ZJ. MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell.* 2011; 146: 448-61.
18. Hu Y, Li W, Gao T, Cui Y, Jin Y, Li P, Ma Q, Liu X, Cao C. The severe acute respiratory syndrome coronavirus nucleocapsid inhibits type I interferon production by interfering with TRIM25-mediated RIG-I ubiquitination. *J Virol.* 2017; 91: e02143-16.
19. Poeck H, Bscheider M, Gross O, et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat Immunol.* 2010; 11: 63–69. doi:10.1038/ni.1824.
20. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature.* 2005; 436: 112-16.
21. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* 2020 Mar 4. pii: S0092-8674(20)30229-4.
22. Ye R, Liu Z. ACE2 exhibits protective effects against LPS-induced acute lung injury in mice by inhibiting the LPS-TLR4 pathway. *Exp Mol Pathol.* 2019; 113: 104350.
23. Zhu W, Yan H, Li S, Nie W, Fan F, Zhu J. PPAR- γ agonist pioglitazone regulates dendritic cells immunogenicity mediated by DC-SIGN via the MAPK and NF- κ B pathways. *Int Immunopharmacol.* 2016; 41: 24–34. doi:10.1016/j.intimp.2016.09.028.
24. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature.* 1998; 391: 82–86. doi:10.1038/34184.
25. Kock G, Bringmann A, Held SA, Daecke S, Heine A, Brossart P. Regulation of dectin-1-mediated dendritic cell activation by peroxisome proliferator-activated receptor-gamma ligand troglitazone. *Blood.* 2011; 117: 3569–3574. doi:10.1182/blood-2010-08-302224.
26. Chen, Y., Niu, Z., Cui, J. et al. The inhibitory effect of troglitazone on macrophage differentiation mediated by repressing NF- κ B activation independently of PPAR γ . *Mol Cell Toxicol.* 2014; 10: 261–268. doi.org/10.1007/s13273-014-0029-4.

27. Hai W, Ping X, Zhi-Wen Y, Chun Z. Therapeutic effect and potential mechanism of pioglitazone in rats with severe acute pancreatitis. *Braz J Med Biol Res.* 2017; 51: e6812. doi:10.1590/1414-431X20176812.
28. Kutsukake M, Matsutani T, Tamura K, et al. Pioglitazone attenuates lung injury by modulating adipose inflammation. *J Surg Res.* 2014; 189: 295–303. doi:10.1016/j.jss.2014.03.007.
29. Zhang W, Xu YZ, Liu B, et al. Pioglitazone upregulates angiotensin converting enzyme 2 expression in insulin-sensitive tissues in rats with high-fat diet-induced nonalcoholic steatohepatitis. *ScientificWorldJournal.* 2014; 2014: 603409. doi:10.1155/2014/603409.
30. Mohapatra J, Sharma M, Singh S, et al. Subtherapeutic dose of pioglitazone reduces expression of inflammatory adipokines in db/db mice. *Pharmacology.* 2009; 84: 203–210. doi:10.1159/000235996.