High-dose intravenous vitamin C treatment for COVID-19 (a mechanistic approach)

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Key words: Sars-CoV-2; Covid-19; Vitamin-C; GAPDH; Macrophage

Abstract
COVID-19 pneumonia seems to be a lung injury caused by the hyperactivation immune effector cells. High-dose vitamin C may result in immunosuppression at the level of these effectors. Therefore, intravenous high-dose vitamin C could be safe and beneficial choice of treatment in the early stages of COVID-19.

Introduction

The two-time Nobel Prize-winning chemist Linus Pauling regarded vitamin C almost as a panacea; therefore, he claimed that high doses vitamin C could combat a host of illnesses, including cancer. He further believed that vitamin C would make the flu disappear completely off the face of the earth.

Coronaviruses (CoVs) are large, enveloped, and positive sense RNA viruses that infect a broad range of vertebrates and cause disease of medical and veterinary significance. Human respiratory corona viruses have been known since the 1960s to circulate worldwide and to cause respiratory infection with rather mild symptoms, suggesting that they are well-adapted to the human host. However, zoonotic coronaviruses, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome coronavirus (MERS-CoV), can cause severe respiratory tract infection with high mortality [1].

Pulmonary pathology during severe coronavirus infection

Primary cell types found in the lower respiratory tract are alveolar epithelial cells and alveolar macrophages (AMs). AMs are not only susceptible to infections, but also release a significant amount infectious virus. Pathological examinations of samples obtained from patients who died of SARS revealed diffuse alveolar damage, accompanied by prominent hyperplasia of pulmonary epithelial cells and presentation of activated alveolar and interstitial macrophages. Strikingly, these pulmonary manifestations were usually found after clearance of viremia and in the absence of other opportunistic infections. Therefore, local inflammatory responses due to excessive host immune response could result in alveolar damage [2].

In a murine model of SARS infection, fast and robust virus replication accompanied by a delayed type I IFN (interferon) response. Accordingly, type I IFN expression was barely detectable in most cell types. Plasmacytoid dendritic cells are a notable exception. They utilize TLR7 (toll-like receptor-7) to sense viral nucleic acids and can induce robust type I IFN expression following coronavirus infection. The extremely rapid replication of SARS-CoV together with the upcoming, but delayed, type I IFN response caused extensive lung inflammation. This was accompanied by influx of inflammatory monocyte-macrophages, which are attracted by inflammatory mediators. Furthermore, macrophages themselves additionally produced high levels of inflammatory mediators through type I IFN stimulation, resulting in further macrophage influx in a pathological feedback loop. Altogether, massive accumulation of pathogenic inflammatory macrophages increased the severity of SARS. Moreover, type I IFN-induced immune dysregulation enforce apoptosis of T cells, which would normally promote virus clearance, resulting in reduced numbers of virus-specific CD8 and CD4 T cells [1, 3].

Activation of effector immune cells

The rapid kinetics of SARS-CoV replication and relative delay in type I IFN signaling may promote inflammatory M1 macrophage accumulation suggesting that targeted antagonism of this pathway would improve outcomes in patients with severe coronavirus infections [2]. Notably, the 2019 novel coronavirus (COVID-19) behaves more like SARS-CoV; accordingly it was named as SARS-CoV-2, progressing rapidly with acute respiratory distress syndrome (ARDS) and septic shock, which were eventually followed by multiple organ failure due to virus-induced cytokine storm in the body [4].

In response to infection macrophages must react rapidly with a substantial pro-inflammatory burst to kill microorganisms and to recruit additional immune cells to infection

site. A sharp increase in the rate of glycolysis is closely associated with inflammatory phenotype in macrophages. Activated macrophages and effector T lymphocytes are shifted to the high glycolytic rate and high glucose uptake, even under oxygen-rich conditions, which is called as "Warburg effect", upon immune activation, similar to cancer cells. Warburg effect is associated with diverse cellular processes, such as angiogenesis, hypoxia, polarization of macrophages, and activation of T cells. This phenomenon is intimately linked to several disorders, including sepsis, autoimmune diseases and cancer [5].

Another interesting aspect of glycolysis induction in activated immune cells is the role of the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). It has been shown that GAPDH binds to the IFNγ coding mRNA, repressing its translation. However, GAPDH dissociates from IFNγ mRNA, allowing to its translation, upon glycolysis activation [6]. In addition, due to the glycolytic pathway stimulation in activated immune cells, their TCA becomes disrupted. Therefore, an accumulation of certain metabolites, including succinate, occurs. Succinate, in turn, may increase hypoxia-inducible factor-dependent activation of target genes, such as IL-1β and GLUT1 [7]. Glucose transporter, GLUT1, is required for the metabolic reprogramming, activation, and expansion of effector lymphocytes and M1 macrophages [7, 8].

Interaction between macrophages and alveolar epithelial type II (ATII) cells

Type I IFNs (type I interferons) produced by almost all type of cells play a vital role in host defense against viral infection and cancer immunosurveillance. In response to viral products pattern recognition receptors, such as retinoic-acid-inducible gene I (RIG-I)-like receptors (RLRs) transmit downstream signaling pathway to trigger type I IFN production in alveolar epithelial cells. Upon sensing cytosolic viral RNAs RLRs undergo conformational changes, oligomerization, and exposure of the CARD domains to recruit a signaling adaptor called mitochondrial antiviral-signaling (MAVS) protein. The transmembrane (TM) domain of MAVS is necessary for its mitochondrial outer membrane localization. Once activated, MAVS develop a functional prion-like structure at mitochondria, leading to the phosphorylation of IRF3 and subsequent transcription and type I IFNs [9].

Activated macrophages produce large amounts of lactate, which are exported by MCT4 [5]. Alveolar epithelial cells import lactate, creating a lactate shuttle between macrophages and ATII cells, and use it as substrate for mitochondrial oxidative energy (ATP) production [10]. In ATII cells, Lactate inhibits MAVS mitochondrial localization, RLR-MAVS association, and MAVS aggregation and downstream signaling activation by binding to the TM domain of MAVS. Thus, macrophage released lactate may attenuate host innate immune response through decreasing type I IFN production for viral clearance [9].

Proposed mechanism of action of high-dose vitamin C in immune effector cells

Vitamin C is known as an essential anti-oxidant and enzymatic co-factor for physiological reactions, such as hormone production, collagen synthesis, and immune potentiation. Humans are unable to synthesize vitamin C; therefore, they must acquire vitamin C from dietary sources [11]. Vitamin C is transported across cellular membranes by sodium vitamin C co-transporter (SVCT). In addition, vitamin C spontaneously oxidizes both intracellularly and extracellularly to its biologically inactive form, dehydroascorbate (DHA) [11, 12]. DHA is unstable at physiological pH and, unless it is reduced back to vitamin C by glutathione (GSH), it may irreversibly be hydrolyzed. Therefore, DHA is reduced to vitamin C after import at the expense of GSH, thioredoxin, and NADPH (reduced nicotinamide adenine dinucleotide phosphate). Consequently, reactive oxygen species (ROS) production

increases inside the activated immune cells (similar to cancer cells) due to the reduction of ROS scavenging systems involving redox couples, such as NADPH/$NADP^+$ and GSH/GSSG (glutathione disulfide). Therefore, high-dose vitamin C, unlike the general assumption, acts as a pro-oxidant in a cell type-dependent manner [12].

Sepsis is characterized by systemic inflammation, increased oxidative stress, insulin resistance, and peripheral hypoxia. Remarkably, severe sepsis resulted in a ~43-fold increase in GAPDH expression [13]. GAPDH is a redox-sensitive enzyme that can become rate-limiting when glycolysis upregulated in the setting of Warburg effect, as it is in both cancer cells [12] and activated immune cells. In addition to oxidizing and inhibiting GAPDH, the elevated ROS may also lead to the DNA damage and the activation of poly(ADP-ribose) polymerase (PARP). PARP activation leads to the NAD^+ (nicotinamide adenine dinucleotide) consumption following vitamin C treatment. Significantly, NAD^+ is required for the enzymatic activity of GAPDH as a co-factor; therefore, the decrease in NAD^+ further diminishes GAPDH enzymatic activity. Altogether, high-dose vitamin C-induced GAPDH inhibition decreases the generation of ATP and pyruvate that induces an energetic crisis (Figure), ultimately leading to cell death [11, 12]. In other words, GAPDH inhibition may lead to the loss of activity of immune effector cells and related immunosuppression. These results provide a mechanistic rationale for exploring the therapeutic use of vitamin C to prevent inflammatory hyperactivation in myeloid and lymphoid cells.

Intravenous high-dose vitamin C treatment for 2019-nCoV disease

The results of meta-analyses have been demonstrated that intravenous (IV) high-dose vitamin C treatment has significant benefits in the treatment of sepsis and septic shock. Sepsis is a life-threatening organ dysfunction syndrome triggered by a disrupting host systemic inflammatory reaction to the pathogenetic microorganisms and their products. ARDS, devastating and mostly lethal condition, is also easily developed in patients with systemic inflammatory response, such as sepsis [14].

Rolipram, a typical phosphodiesterase-4 inhibitor, can inhibit TNFα production in activated macrophages and restrain acute inflammatory response. Rolipram was suggested as a novel drug treatment for sepsis and septic shock due to its potent immunosuppressive effects [15]. By analogy, the beneficial effects of intravenous high-dose vitamin C in sepsis and septic shock are most likely due to its immunosuppressive effects.

While immune effector cells are dependent on glycolysis for their bioenergetic functions, lung epithelial cells use mitochondrial oxidative phosphorylation to produce ATP. Therefore, high-dose vitamin C treatment acts as a prooxidant for immune cells, but as an antioxidant for lung epithelial cells. Furthermore, vitamin c treatment may protect innate immunity of ATII through the inhibition of the lactate secretion, produced by the activated immune cells.

In connection with the prooxidant role of vitamin C, which requires pharmacological (millimolar) rather than physiological (micromolar) concentrations, reevaluating the high-dose infusion of vitamin C would be a timely choice for the COVID-19-related ARDS. Altogether, patients diagnosed with COVID-19 and hospitalized with the breathing difficulty and abnormal biomarkers seem to be candidate for a short period of high dose intravenous vitamin C treatment in the early periods of the disease. However, the concern that may arise with high-dose vitamin c treatment is osmotic cell death of immune cells, but not apoptosis, which could generate a local inflammation in alveolar medium. Therefore, IV glucocorticoid treatment must be added to attenuate the possible inflammatory complications of high-dose vitamin c treatment. Previously experienced and comparably well-tolerated treatment regimen for high-dose intravenous vitamin C could be the administration

of 50 mg/ per kilogram body weight every 6 hours for 4 days [14] with a glucose restriction. In addition, hydrocortisone 50 mg IV every 6 hours for 7 days must be added to fight against therapy-induced inflammation. Vitamin C when used as a parenteral agent in high doses may act pleiotropically as a prooxidant to attenuate pro-inflammatory mediator expression, improving alveolar fluid clearance, and to act as an antioxidant to improve epithelial cell functions.

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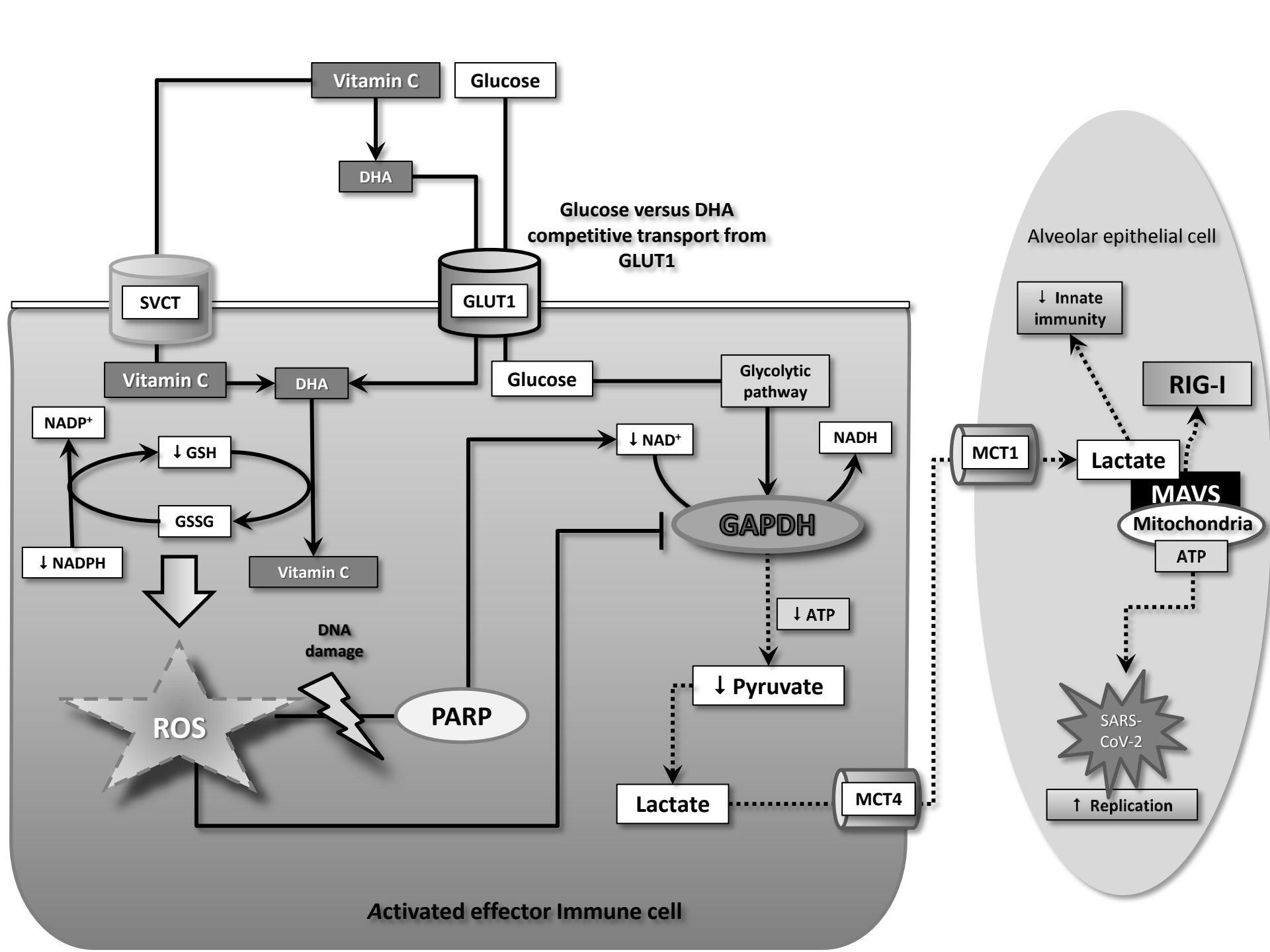
Vitamin C
Glucose
DHA
Glucose versus DHA
competitive transport from
GLUT1
SVCT
GLUT1
Vitamin C
DHA
Glucose
Glycolytic
pathway
NADP+
↓ GSH
GSSG
↓ NADPH
Vitamin C
↓ NAD+
NADH
GAPDH
↓ ATP
↓ Pyruvate
DNA
damage
ROS
PARP
Lactate
MCT4
Activated effector Immune cell
Alveolar epithelial cell
↓ Innate
immunity
RIG-I
MCT1
Lactate
MAVS
Mitochondria
ATP
SARS-
CoV-2
↑ Replication