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Review

The role of iron in the pathogenesis of COVID-19 and possible treatment with lactoferrin and other iron chelators

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ABSTRACT

Iron overload is increasingly implicated as a contributor to the pathogenesis of COVID-19. Indeed, several of the manifestations of COVID-19, such as inflammation, hypercoagulation, hyperferritinemia, and immune dysfunction are also reminiscent of iron overload. Although iron is essential for all living cells, free unbound iron, resulting from iron dysregulation and overload, is very reactive and potentially toxic due to its role in the generation of reactive oxygen species (ROS). ROS react with and damage cellular lipids, nucleic acids, and proteins, with consequent activation of either acute or chronic inflammatory processes implicated in multiple clinical conditions. Moreover, iron-catalyzed lipid damage exerts a direct causative effect on the newly discovered nonapoptotic cell death known as ferroptosis. Unlike apoptosis, ferroptosis is immunogenic and not only leads to amplified cell death but also promotes a series of reactions associated with inflammation. Iron chelators are generally safe and are proven to protect patients in clinical conditions characterized by iron overload. There is also an abundance of evidence that iron chelators possess antiviral activities. Furthermore, the naturally occurring iron chelator lactoferrin (Lf) exerts immunomodulatory as well as anti-inflammatory effects and can bind to several receptors used by coronaviruses thereby blocking their entry into host cells. Iron chelators may consequently be of high therapeutic value during the present COVID-19 pandemic.

1. Introduction

Coronavirus disease 2019 (COVID-19), triggered by the SARS-CoV-2 virus, is an enormous health concern and can be profoundly detrimental. The COVID-19 epidemic is calling for both national and international attention to develop effective therapeutics, including selective vaccines. However, no specific therapies presently exist, leaving the patients to depend on general and supportive therapies, including oxygen supply and broad-spectrum antiviral medications such as IFN-α (interferon-α), human serum albumin (HSA), and glucocorticoid [1]. Most currently, lopinavir/ritonavir, a permitted anti-HIV drug, has been recommended for the treatment of COVID-19 infection [2]. Remdesivir, a novel nucleotide analog prodrug in development for treating Ebola virus and Middle East Respiratory Syndrome (MERS) diseases, has also been reported to relieve the pneumonia symptoms of COVID-19 infection. While these drugs exhibit promising results, further therapeutic options should be discovered and evaluated thoroughly when considering the increasing number of COVID-19 cases.

COVID-19 manifests itself in many complications as well as physiological and biochemical alterations. These include, but are not limited to, Acute Respiratory Distress Syndrome (ARDS) [3], high concentrations of proinflammatory CD4 T cells and cytotoxic granules CD8 T [4], massive release of cytokines (cytokine storm) [5], increased coagulation state [6], hemoglobin damage [7] and dysregulation of iron homeostasis [8] including iron overload [9,10] which is likely a major factor in the pathogenesis of COVID-19.

Liu, et al. (2020) [7] suggest that the key pathogenic molecular step of COVID-19 is to attack hemoglobin causing dissociation of the porphyrins from iron and releasing iron into the circulation. Thus, hemoglobin loses its capacity to bind with oxygen and hinders its delivery to major organs, which is coupled with rapid multi-organ failures. Moreover, the free iron released into the circulation may result in iron overload causing oxidative damage to the lungs and other organs. Iron overload may also result in inflammation and immune dysfunction [11,12]. These dictate increased uptake and storage of iron in iron-binding proteins. Indeed, this notion is supported by the increased ferritin (the

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iron storage molecule in the body) concentrations in the circulation of COVID-19 patients [9,10]. Amplified iron load leads to increased blood viscosity with recurrent and diffuse macro and micro circulatory thrombosis; this may explain the cause of unexpected deterioration and death in some cases [7,13,14].

Thus, we can consider iron chelation as a beneficial adjuvant therapy in treating COVID-19. Iron chelators, such as deferasirox, deferoxamine, and deferiprone, in addition to the naturally-occurring iron chelator lactoferrin, may prove to be effective in the fight against COVID-19 [15]. Iron chelators may have a role to play, not only with sequestering iron and alleviating inflammation but also in preventing the coronavirus from binding to receptors it utilizes for entry into host cells [16–18].

2. Pathology of COVID-19

2.1. Acute respiratory distress syndrome

Acute Respiratory Distress Syndrome (ARDS) is a common complication of severe viral pneumonia, including pneumonia caused by highly pathogenic coronaviruses such as SARS-CoV-2 [19]. ARDS is a life-threatening lung condition that prevents sufficient oxygen supply to the lungs accounting for the majority of deaths associated with respiratory disorders and acute lung injury [20]. In fatal cases of human SARS-CoV, MERS-CoV, and SARS-CoV-2 infections, individuals exhibit severe respiratory distress requiring mechanical ventilation, with histopathology findings also supporting ARDS [4,21,22]. Earlier studies have found that genetic susceptibility and inflammatory cytokines were closely linked to the occurrence of ARDS. Over 40 candidate genes including interleukin 10 (IL-10), ACE2, tumor necrosis factor (TNF), and vascular endothelial growth factor (VEGF) are considered to be associated with the development or outcome of ARDS [23]. Increased levels of plasma IL-6 and IL-8 were also demonstrated to be related to adverse outcomes of ARDS [20]. The above biomarkers suggest both a molecular explanation for the severe ARDS and a possible treatment for ARDS following SARS-CoV-2 infection.

2.2. Cytokine storm

The virus replicates within the host cells once fusion is completed. This invasion of lung surface cells directly causes lung inflammation and a detrimental cycle of oxidative stress-mediated functions including PARP and PARG activity, ADP ribose increase, and TRPM2 activity [24]. After virus-cell interaction, the antigen-presenting cells (APCs), including macrophages, present SARS-CoV-2 antigens to T cells after its entrance into the host cells. This process leads to T cell activation and production of cytokines in various T cell subsets, i.e., Th17, tracked by a massive release of cytokines due to a positive response loop among cytokines and immune cells. On the other hand, during SARS-CoV-2 replication, the virus genomic dsRNA activates interferon regulatory factors (IRFs) and TLR-3-induced NF-κB pathway, which culminates in the production of type I IFNs and proinflammatory cytokines in large quantities [25]. Moreover, increased free iron and hyperferritinemia resulting from iron dysregulation and overload in COVID-19 patients may exacerbate inflammatory processes through ROS-induced oxidative damage of cellular biomolecules [26], and possibly immunogenic ferroptosis [26]. This is discussed in more detail in Section 2.4. and Section 3. These mechanisms may not only result in uncontrolled endothelial dysregulation of immune responses following hyper-inflammation and cytokine storms, but also may lead to multiple organ failure, pulmonary tissue damage, and reduced lung capacity [5,8]. Additionally, infection with SARS-CoV-2 causes a significantly amplified level of plasma pro-inflammatory cytokines [24,27] including MIP1α, MCP1, MIP1β, IL1RA, IL1-β, IL7, IL8, IL9, IL10, IP10, PDGFB basic FGF2, GMCSF, GCSF, TNFα, IFNγ, and VEGFA [24,27].

2.3. Hypercoagulation

Thromboembolic complications were reported by several recent studies in severe COVID-19 patients [28–33]. However, the exact causes of these events are still unknown. Inflammation-induced coagulation, which leads to an increased coagulation state, is typically instigated through tissue factor (TF/CD142/coagulation factor III) [6,34]. TF is expressed on both mononuclear cells in response to pro-inflammatory cytokines (mainly IL-6) as well as on vascular endothelial cells [35]. It is utilized in advancing the conversion of prothrombin into thrombin, which successively transforms circulating fibrinogen into fibrin, resulting in fibrin-based blood clots [35]. Moreover, inflammation is known to impair major natural anticoagulant pathways, such as antithrombin or TF pathway inhibitor, furthering the dissemination of coagulation. Employment of TF-expressing inflammatory monocytes by activated endothelial cells is entirely responsible for coagulation initiation if vascular injury is absent [36]. Oxidized phospholipids (OxPLs), which have been present in the lungs of SARS-CoV-2 patients, may also contribute to the process [6]. This is theorized due to evidence of OxPLs production after oxidative stress; they promote the initiation of TF expression and inflammatory programs in monocytes, as well as activate endothelial cells to recruit and bind to monocytes [37]. Hyperferritinemia and free unbound iron may also contribute to hypercoagulation observed in COVID-19. This is discussed in Section 2.4 and Section 3, respectively.

2.4. Hyperferritinemia

Many COVID-19 patients with a raised serum ferritin level of >300 μg/l had a 9-fold increase in the chances of death before discharge [6,9]. Hyperferritinemia is largely considered an indicator of the “hyperferritinemic syndromes” associated with severe COVID-19. This condition characterizes several autoimmune diseases [38] and due to its immunomodulatory properties may play a pathogenic role [39]. There has also been increasing evidence indicating that high circulating ferritin may not only display an acute phase response but may also be crucial to inflammation [39]. Ferritin is an important intracellular iron storage protein that consists of two subunits, H and L, that vary in proportion by tissue type and physiologic status of the cell [40]. Although circulating serum ferritin’s source during inflammatory conditions is still uncertain, in vitro experiments provide the possibility of it being secreted through hepatocytes [41] and by macrophages through a nonconventional pathway [42]. This demonstrates the likelihood of a contribution to ferritin production through macrophage activation, in “hyperferritinemic syndromes”. Moreover, a previous study illustrated AOSD ferritin serum levels to be related to both disease activity as well as macrophage activation [3]. Another study also provided a cohort of 39 COVID-19 patients who exhibited ferritin serum levels that significantly correlated to disease severity [19]. Another major trait of serum ferritin, besides an active secretion during an inflammatory response, is the death of hepatic cells. After release, serum ferritin loses part of the inner iron content, producing extremely high levels of free iron [43]. The overabundance of free iron detected during severe inflammatory conditions can deteriorate the inflammatory reaction; particularly inducing a marked pro-coagulant state [43]. It is also argued that the COVID-19 virus may exhibit hepcidin-mimic effects, which might result in increased ferritin level independent of the inflammatory response and increase the risk of coagulopathy [26].

2.5. Hemoglobin damage

The pulmonary inefficiency detected in COVID-19 patients may not be caused by cell damage in the lungs alone; this is supported by the atypical presentation of ARDS [46–48]. Liu and Li [7] have recently studied the pathogenesis of SARS-CoV-2 and have obtained evidence indicating its key pathogenic strategy. This strategy involves attacking

the 1-beta chain of hemoglobin, which consequently initiates dissociation of porphyrins from iron and discharging of iron into the circulation. As a result, hemoglobin's ability to bind with oxygen is destroyed, negatively affecting its delivery to the main organs of the body. This reveals why severe hypoxia is not altered with oxygen and instead is related to rapid multi-organ failures. Furthermore, besides inducing oxidative damage of the lungs, free iron causes inflammation of alveolar macrophages as well. Disruption of the heme pathway can result in amplified serum ferritin levels, lymphopenia, and elevated monocytes. Additionally, increased iron load and hemoglobin production contribute to higher blood viscosity with recurrent and diffuse macro and micro circulatory thrombosis. This leads to elevated levels of D-dimer, which may justify the cause of sudden deterioration and death in some cases [7,13,14].

Liu & Li [7] reported, based on recently published studies, that postmortem analysis revealed pulmonary thrombosis instead of typical ARDS. They concluded that COVID-19 does not cause pneumonia or ARDS. The virus structural protein may instead bind to hemoglobin, shifting oxygen and iron [7]. As mentioned previously, the free iron leads to inflammation and the toxicity of alveolar macrophages. The toxicity associated with free iron is so high that it causes oxidative damage to the lungs, which explains the bilateral - ground-glass opacities seen on the chests of those patients by CT. The authors claim that this condition was incorrectly treated as bilateral pneumonia. Lung inflammation starts due to the inability of both carbon dioxide and oxygen exchange. Hemoglobin loses its capacity to bind with oxygen which generates oxygen deficiency in major organs, leading to resistant hypoxia with concomitant rapid multi-organ failures. Invasive ventilation does not benefit these patients, meaning they may require recurrent blood transfusions. Their body attempts to recompense by elevating the rate of hemoglobin synthesis, which explains the high levels of Hb detected in these patients. Another compensatory mechanism observed is to deal with the iron load through boosting ferritin production. This may explain the very high ferritin observed in those patients [7].

2.6. Blood groups and COVID-19

Interestingly, increasing evidence is rapidly emerging on the association between blood groups and COVID-19 infection, with blood type A individuals being at the highest risk and those of blood type O at the lowest risk for developing the disease [49–54]. Dai, 2020 [55] reported that individuals having an O blood group type are less likely to develop cardiovascular diseases and severe COVID-19 and, on the contrary, patients carrying an A blood group type, especially those already having been diagnosed with cardiovascular diseases, in particular hypertension, are more likely to develop severe COVID-19 once infected. Ellinghaus, et al. 2020 [56] studied 1610 COVID-19 positive patients with respiratory failure and reported that the genetic data confirm that blood group O is associated with a lower risk of acquiring COVID-19 than that in non-O blood groups, whereas blood group A was associated with a higher risk than non-A blood groups.

Although the relationship between blood groups and COVID-19 is not yet clear, the blood iron status can provide a link between them. A few studies reported that some serum iron indicators are lower in individuals with blood group O compared to those with other blood groups [57–63]. Higher amounts of iron may contribute to multiple complications associated with COVID-19, including inflammation and hypercoagulation. The relationship between iron and COVID-19 is discussed in detail in the following section.

3. Iron and inflammation

Iron is an essential element for all living cells as it is key to establishing many functioning metabolic processes including DNA synthesis, DNA repair, DNA transcription, energy production, oxygen transport, oxygen storage, and drug detoxification [64]. Through executing gain and loss of electrons via redox-fluctuation between divalent ferrous (Fe^{2+}) and trivalent ferric (Fe^{3+}) ions (which make up the iron pool (IP) in the intracellular environment), iron is easily distinguished as critical to biological functions [64]. Nevertheless, free unbound Fe^{2+} is characterized by a high reactivity and potentially high toxicity that can be ascribed to the induction of ROS formation by the Fenton and Haber-Weiss reactions [64]. ROS formation, which causes lipid, nucleic acid, and protein destruction, is involved in cell, tissue, and organ oxidative stress and injury, with consequent activation of acute/chronic inflammatory processes connected to various degenerative clinical conditions [64].

Iron-catalyzed lipid peroxidation by Fenton reactions has been increasingly implicated in the newly discovered non-apoptotic cell death known as ferroptosis [65]. Ferroptosis, dissimilar to the immunologically silent apoptosis, induces an immune response when affected cells release damage-associated molecular patterns (DAMPs) and alarmins, which amplify cell death and promote a series of reactions related to inflammation [66–69]. Evidence verifying ferroptosis as contributing positively to inflammation has increased immensely. Additionally, there have been studies displaying the ability of several compounds to behave like ferroptosis inhibitors through the execution of anti-inflammatory effects [65].

There are two main methods by which iron homeostasis is maintained in mammals: dietary iron absorption from the enterocytes (1–2 mg) and iron recycling through the lysis of senescent erythrocytes by macrophages (about 20 mg/day) [64,70]. In the enterocyte cytoplasm, ferritin isolates and oxidizes ferrous ions belonging to the IP [64]. Now on the basolateral side of enterocytes, in order to transport iron out from the cells to the blood, the protein ferroportin is required. This protein plays a major role in the releasing Fe^{2+} to hephaestin (Heph). Heph then can oxidize the iron to initiate binding of Fe^{3+} to transferrin (Tf), a glycoprotein capable of binding two ferric ions per molecule, in the blood. This glycoprotein is crucial in the iron transport process from blood to cells, and iron is then freed from Tf into the cytoplasm [71]. Here, the iron is either used in cell metabolic processes or isolated by ferritin and exported by ferroportin teaming with ceruloplasmin (Cp), an alternative ferroxidase similar to Heph [72].

Hepcidin is a hormone constructed from a 25 amino acid cationic peptide hormone [70]. It is a common regulator of iron homeostasis, is modulated by iron stores as well as by hypoxia, plays a vital role in internalizing and degrading ferroportin, and is primarily found in plasma and urine [70,73]. Elevated levels of this hormone, which can impair iron export by ferroportin, results in intracellular iron overload and a reduction of iron import systems [74]. On the other hand, low levels of hepcidin prevent the activation of ferroportin degradation and restore iron export as well as iron import [70,75]. When there are inflated levels of pro-inflammatory cytokines, such as IL-1α, IL-1β, and IL-6, present during inflammation, both hepcidin up-expression and ferroportin down-regulation significantly impede iron export resulting in intracellular iron overload [64].

3.1. Iron and lung

Elevated iron concentrations in the lung are associated with an increased risk of pulmonary injury [76,77]. In turn, both acute and chronic lung injury also lead to disruption of iron homeostasis within the lungs [77]. Changes in oxygen saturation levels (hyperoxia and hypoxia) modify iron metabolism and can severely injure the lungs, considering the organ's high susceptibility to metal-induced oxidative stress [76]. Lung injury is characterized by severe hypoxemia, increased endothelial and epithelial permeability, increased cytokine levels in the lungs, and neutrophilic alveolar infiltrates [78].

As mentioned previously, ARDS consists of lung damage through endothelial activation and capillary membrane destruction, allowing protein leakage [79]. Iron intensifies this inflammatory lung injury by combining superoxide and hydrogen peroxide from ARDS with iron's

ability to catalyze more toxic reactive oxygen species [76]. High serum ferritin is also heavily associated with ARDS progression [76,80]. As iron levels begin to increase, ferritin also increases isolating reactive iron. Additionally, synthesis of ferritin (being it is an acute reactive protein) is elevated during the inflammatory response and increased levels may be due to increased tissue damage and lysis [80]. Lavage fluid of ARDS patients showed elevated levels of total and nonheme iron as well as cellular content of Tf, ferritin, and Lf [81], suggesting disruption of pulmonary iron homeostasis in ARDS [76].

3.2. Iron and thrombosis

A recent study by Lipinski and Pretorius 2013 [82], observed the levels of free iron in the blood that contribute to non-enzymatic generation of parafibrin. Parafibrin is an insoluble biomaterial, similar to fibrin, that posseses the ability to trigger inflammatory reactions in the arteries after deposition. The study found that free iron in the blood is able to produce hydroxyl radicals in order to convert fibrinogen into fibrin clots. Iron chelators, discussed in more detail in later sections, can be used to inhibit this process and scavenge hydroxyl radicals. Gill et al. [83] concluded that iron has been a fundamental factor in multiple aspects of pathological thrombosis, including oxidative stress, thrombocytosis, and increased erythrocyte viscosity. A third study conducted by Benyamin et al. [84] stated that high levels of iron presented an elevated risk of venous thromboembolism. Therefore, based on all these studies, a reasonable conclusion suggests that iron overload must play a role in hypercoagulation found in severe COVID-19 patients.

4. Lactoferrin

Lactoferrin (Lf) is a highly abundant breast-milk glycoprotein that is produced by various mucosal epithelial cells. It has a molecular weight of 80 kDa [15,85] and is usually found in various secretory fluids, like tears, saliva, and the nasal secretions of different mammalian species, including humans, camels, cows, dogs, goats, horses, and numerous rodents. Furthermore, Lf has been identified in the eggs of rainbow trout, indicating that this protein is also produced by fish [86]. Lf remains an essential player of the usual natural immunity. It is abundant in the lower and upper lung tissues in addition to the gastric and urogenital mucosal tissues, which are frequently exposed to the colonizing or aggressive microbial pathogens. It is broadly distributed in specific neutrophil granules of polymorphonuclear leukocytes as well as biological fluids, such as milk, saliva, and seminal fluid [87]. Lf displays a broad range of preventive, therapeutic, and biological activities, including antifungal, antibacterial, antiviral, anticancer, antioxidant, immunomodulatory, cell-growth modulatory, and binding functions, in addition to neutralizing some bioactive substances, such as lipopolysaccharide (LPS) and glycosaminoglycan [88–92]. Additionally, Lf is a member of the transferrin family and has a higher affinity to bind to iron than transferrin [93]. FDA considers Lf as 'generally regarded to be safe" (GRAS) with no contraindications. It is extensively used as a nutritional additive in infant formulas and clinical studies that employed Lf doses from 100 mg to 4.5 g a day, for numerous conditions, found no apparent toxicities [15,94].

4.1. Lf structure

The structure of Lf consists of two homologous globular lobes (N-lobe and C-lobe) (Fig. 1), which are further split into two domains (N1 and N2) and (C1 and C2). Each lobe has Fe-binding sites located in the interdomain clefts [95] that are identical to the other lobe and allow them to bind two Fe^{3+} ions. CO_3^{2-} ions also simultaneously bind to each of these iron molecules. However, the sites are individually made up of four amino acid ligands (2 Tyr, 1 Asp, and 1 His) as well, which have three negative charges to counteract the positive charges of Fe^{3+}. A helix N-terminus and Arg side chain consist of positive charges to balance the negatives ones obtained from the carbonate anion. Additionally, there is an inter-lobe region comprising 11 residues in the form of a 3-turn α-helix [96].

4.2. Lf Iron binding mechanism

The iron-binding mechanism of Lf has been studied extensively. The overall iron-binding mechanism works cooperatively, where initially the C-lobe binds with Fe^{3+} stimulating the N-lobe for iron-binding subsequently. Next, inter-domain hydrogen bonding works to enclose the Fe^{3+} molecule in the iron-binding domains, by strategically occurring at the lips of the iron-binding cleft [16,97]. Normally, Lf has an iron

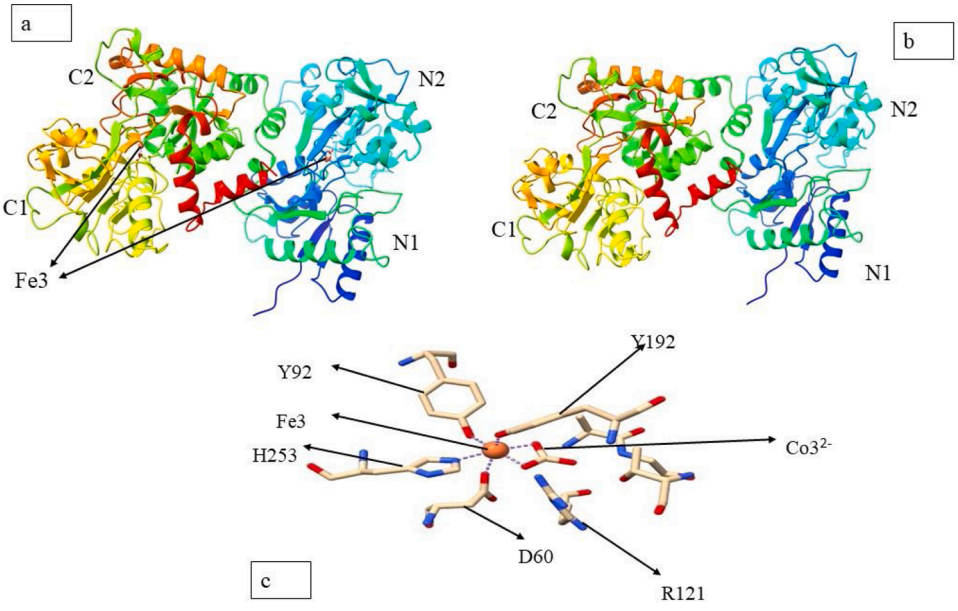

Fig. 1. (a) Structure of lactoferrin in holo-form (iron-saturated); (b) Structure of lactoferrin in apo-form (iron-free); and (c) Lactoferrin iron-binding site: two tyrosine (Y92 and Y192), one aspartic acid (D60), one histidine (H253) and one carbonate anion together with the arginine residue (R121). Modeled using Chimera software (http://www.cgl.ucsf.edu/chimera/). And PDB id 1BLF.

saturation of about 15–20 % and is considered only partially saturated. Iron-depleted Lf (which displays <5% iron saturation) is called apo-Lf whereas saturated Lf is known as holo-Lf. As iron-binding produces a structure where each lobe encompasses the bounded iron ion, holo-Lf has a compact form where Fe^{3+} is effectively isolated from the environment. Conversely, apo-Lf exhibits lower stability and density than holo-Lf; this means any metal binding or release results in significant conformational transformation [95].

4.3. Lf Iron release mechanism

Three factors are responsible for structural changes required for iron discharge: the presence of specific receptors like that in serum transferrin, reduction of Fe^{3+} to Fe^{2+} (Lf has a significantly lower affinity to Fe^{2+}), and a decrease in pH of the environment. Taking the latter into consideration, crystallographic and iron release kinetics have been utilized to thoroughly observe the pH-triggered release of iron. The carbonate ion, tyrosine, and/or histidine ligands present within the iron-binding domain are protonated at certain low pH values dependent on the source of Lf. Dropping the pH as such diminishes iron coordination to the point where it is unable to hold the two Lf domains together. Afterward, the iron-binding domains start to open and interact with existing positive charges in the environment. This eventually promotes the release of iron [16,97]

4.4. Lf in the immune system

Lactoferrin is capable of immunomodulation on adaptive cellular functions of both T and B lymphocytes as well as other immune cells. This is conducted by stimulating both the maturation of T-cell precursors into adept helper cells and the differentiation of immature B-cells into efficient antigen-presenting cells (APCs) [85]. Most cytokines perform as early mediators of effector T-cell development [98], partly due to the strength in the relation between cellular and humoral immune responses. Recent research has concluded that lactoferrin can display immunoregulatory effects on Th1/Th2 cell activities and initiate T-cell differentiation from their immature precursors through inducing the CD4 antigen expression [99,100]. Additionally, many studies have revealed a vital relationship between Lf and CD4+T-cells. Due to this relationship being the main focus of studies, Lf regulation of Th1/Th2 cytokines based on host immune response, in diseases, has also been studied immensely. Here, Lf can result in Th1 polarization, a state over which regulating infection or tumors depends on a strong Th1 response [100]. By the activation of macrophages and the creation of reactive oxygen intermediates, Th1 cells are given the ability to progress with intracellular killing events that extinguish pathogens [101]. Lactoferrin receptors are expressed on all T-cell subsets, with $\delta\gamma$ T-cells. Additionally, Lf can connect to human T-cell surface receptors [95] and receptor-mediated endocytosis may play a role in Lf binding. Wang et al [16] reported that Lf elevated the lymphoid and resident intestinal CD4+and CD8+T-cells in mice implanted with tumor cells. Moreover, Lf modulates the T-cell activity and their reactivity to local antigens [95] as well as encourages cellular recruitment which triggers APC activation, and consequently enhances antigen-specific immune responses. Therefore, it is unsurprising that Lf is regarded as an authentic alarmin that reinforces a Th1-type immune response. T-cell populations can also be affected by Lf; this occurs through a particular targeting of a subset of cellular responses. A well-researched phenomenon is the ability of Lf to directly change the balance between the Th1 and Th2 immune responses, frequently defined by the T-cell cytokines IFN-g and IL-4/IL-5, respectively. As with the general T-cell response, Lf additionally affects both Th1 and Th2 cellular subsets. It can also promote B-cells development by increasing complement 3 receptor (C3R) expression, advancing the acquisition of surface IgD and enhancing B-cell antigen presentation [85].

4.5. Lf in the inflammation system

Contemporary in vivo and in vitro research has proven the existence of several anti-inflammatory mechanisms by which lactoferrin acts. These mechanisms range from modulation of cytokine/chemokine production to regulating the production of ROS and immune cell recruitment [102,103]. Lactoferrin is an iron scavenger and an activator/regulator of signaling pathways. As such, it brings about negative feedback on the inflammatory response which is indicated by a reduction in both the generation of ROS and several pro-inflammatory cytokines [104–106]. Several inflammatory disorders increase the concentration of Lf, including neurodegenerative diseases, inflammatory disease, arthritis, and allergic inflammation [104].

4.6. Lf in lung inflammation

The most relevant lung damages are observed in airways that are characteristically susceptible to infections of cystic fibrosis (CF). Significant airways inflammatory response is usually observed in CF subjects; observed through high levels of IL-8 and neutrophil accumulation in bronchoalveolar lavage (BAL) [107]. In general, the inflammatory status is activated by an over-expression of NF-κB and activator protein (AP)-1 leading to an up-expression of pro-inflammatory cytokines [108, 109]. The inflammatory response begins a vicious cycle when leukocytes are enabled and enter the lung airways; the infiltrated neutrophils experience necrosis and begin to let out chemoattractant molecules and proteases, which eventually result in tissue injury as well as induce additional leukocytes to travel to the area.

Frioni et al. [110] reported that the introduction of bovine Lf (bLf) to a primary human CF airway epithelium resulted in a reduced inflammatory response by infected bronchial epithelium through down-regulation of IL-1β, IL-6, and IL-8 levels. Also, Valenti, et al. [111] reported that mice treated with Lf showed a significant decrease of total leukocytes and neutrophils counts in BALs for both acute and chronic infection models, compared to saline-treated ones. Furthermore, Lf was effective in differently reducing the expressions of numerous pro-inflammatory cytokines, including macrophage inflammatory protein (MIP-1α), IL-1α, β, 6, KC (similar to human IL-8), (p40), granulocyte-colony stimulating factor (G-CSF), IL-12 and IL-12 (p70), depending on the acute or chronic infection. Additionally, the bLf-mediated reduction of molecules related to neutrophil development and migration (KC, G-CSF, and MIP-1α) was shown to relate to a reduction in neutrophil recruitment [64,110,112].

4.7. Lf antiviral activity

Lf has been found to impede the activities of, both naked and enveloped, DNA and RNA viruses such as cytomegalovirus, herpes simplex virus, human immunodeficiency virus (HIV), rotavirus, poliovirus, respiratory syncytial virus, hepatitis B and C (HCV) viruses, parainfluenza virus, alphavirus, hantavirus, human papillomavirus, adenovirus, enterovirus 71, echovirus 6, influenza A virus, and Japanese encephalitis virus [15,16]. Lf also possesses the capability of obstructing viral entry, perhaps through binding to viral particles, cell surface molecules, or both.

Research studies, conducted over recent years, have found the process of viral entry to be based on complexities involving cell surface molecules. This occurs through virus attachment proceeded by binding to a high-affinity cell surface receptor [15,16]. In order to effectively bind to the specific entry receptor (ACEII), viruses engage with adhesion molecules known as heparan sulfate proteoglycans (HSPGs). These molecules ensure specific viral binding to ACEII by allowing the virus to increase in concentration at the cell surface. That being said, Lf has been shown to oppose this by binding to HSPGs which allows for the prevention of viral entry [113,114]. Additionally, Lf is also effective in binding directly to virus particles, as in HCV, to redirect them away from

their initial target cells [85]. Its potential is further evident in Lf's ability to repress viral replication after entry of the virus (as in HIV) and its capability of producing an indirect antiviral effect upon immune cells. This makes Lf a paramount line of defense at the initial stages of an infection [115]. Fig. 2 is an illustration of the coronavirus cell entry and the protective role of lactoferrin in coronavirus infection.

5. Iron chelators

5.1. Iron chelators and iron overload

Iron overload is a condition that elevates the amount of non-transferrin bound iron (NTBI), which is essentially free iron in the plasma. Disturbance of various cellular organelles and tissue deterioration, including that of the liver, lung, and heart, can be the result of NTBI collection, which leads to an upsurge in free iron entering into cells [116–118]. Furthermore, an increase in iron within the heart may cause iron overload cardiomyopathy; a frequent cause of mortality in these patients [116,119]. Iron overload cardiomyopathy arises as a patient suffers from iron overload and free iron starts to infiltrate the heart through T-type calcium channels and L-type calcium channels [120] When there is an excessive amount of free iron within the cell, the free iron starts to produce a toxic hydroxyl radical($^{\bullet}OH$) by reacting with hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$), via Haber-Weiss and Fenton's reactions; this results in cardiac iron toxicity [121]. The creation of the harmful hydroxyl radical elevates ROS levels as well as cardiac oxidative stress, which conclusively impairs cardiac cells and gives rise to cardiac dysfunction and heart failure [121] Furthermore, under iron overload circumstances, rise in ROS production is followed by plasma membrane lipid peroxidation which also produces cytotoxic malondialdehyde through Haber-Weiss and Fenton's reactions [122]. This product, similar to the hydroxyl radical, is extremely dangerous to cells, consequently displaying cardiac dysfunction in one murine model. Malondialdehyde has also been observed to be largely present in cardiac iron overload, with studies showing it to be elevated in plasma and tissue of iron-overloaded rats [123,124]. The chemicals utilized in the Haber-Weiss and Fenton's reactions that react with free iron, hydrogen peroxide, and superoxide, are found to originate from the mitochondrial electron transport chain [121] Thus, iron-catalyzed oxidants have been found to result in mitochondrial dysfunction and mitochondrial DNA damage; resulting in cardiac dysfunction and a loss of respiratory capacity as well [125].

Patients with iron overload are typically treated with iron chelators such as deferasirox, deferoxamine, and deferiprone. Iron chelators possess multiple toxicological properties that contribute to their effective application, such as chelating iron, inhibiting redox properties exerted by free iron, and averting the participation of iron in Fenton reactions. Their role in hindering Fenton reactions allows inhibition of hydroxyl radical production as well as the creation of other ROS that inflict oxidative damage and ferroptosis [17]. Ferroptosis is known to be a type of controlled cell death [126] that has characteristics relating to elevated free iron levels associated with ROS [127]. Additionally, decreases of glutathione and/or alterations of glutathione peroxidase 4 (GPX4), which functions as a ROS regulator, are created in response to ferroptosis [127–129].

In addition to binding with free iron, there are other possible mechanisms by which iron chelators can act as potential therapeutic options in COVID-19. These mechanisms include downregulation of hepcidin [130] and removing iron from iron-binding proteins, thus, exerting anti-ferritin effects and attenuating the "hyperferritinemic syndromes" associated with severe COVID-19 [131]. The properties of the mentioned iron chelators are shown in Table 1[132,133], chemical structures are presented in Fig. 3, and the mechanism of action is illustrated in Fig. 4.

Table 1
Iron chelator properties. [ab.]

	Deferoxamine	Deferiprone	Deferasirox
FDA approval	1968	2011	2015
PubChem CID	2973	2972	214,348
Molecular weight	560.7 g/mol	139.15 g/mol	373.4 g/mol
Molecular Formula	$C_{25}H_{48}N_6O_8$	$C_7H_9NO_2$	$C_{21}H_{15}N_3O_4$
Administration	IV, IM, SQ	Oral	Oral
Elimination half-life	6 h.	2 to 3 h	8 to 16 h
Excretion	Renal	Renal (75 to9 0%) in 24 h	Fecal (84 %) & renal (8%)
Dosing frequency	Daily for 5–7 days/week	3-time Daily	1 Daily
Dose	1000 – 2000 mg/kg	75 mg/kg	14 mg/kg
Adverse effects	Infusion site reactions, gastrointestinal disturbances, renal insufficiency	Gastrointestinal disturbances, liver function abnormalities, arthralgia, neutropenia	Gastrointestinal disturbances, renal insufficiency, rash, liver function abnormalities

SQ: subcutaneous, IV: intravenous, IM: intramuscular [a]PubChem [132] (National Center for Biotechnology Information. PubChem Database. https://pubchem.ncbi.nlm.nih.gov/compound/.: [b] (Shammo & Chalmers, 2016) [133].

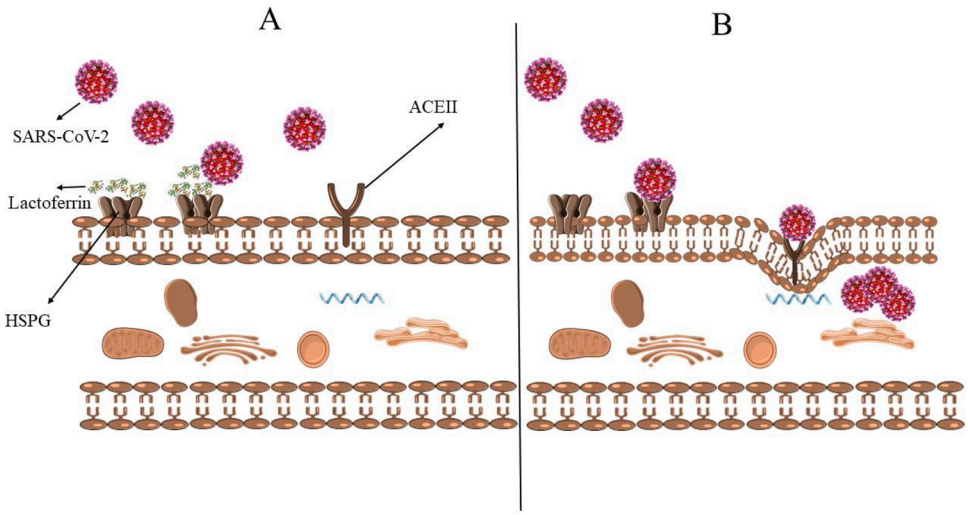

Fig. 2. A model of coronavirus cell entry and the protective role of lactoferrin in coronavirus infection. A: Lf blocks the infection of coronavirus by binding to HSPGs. Lactoferrin expression may be up-regulated when the coronavirus infects the human body. Lactoferrin locates to cell-surface HSPGs and prevents the preliminary interaction between the virus and host cells and the subsequent internalization process. B HSPGs play an important role in the process of coronavirus cell entry. The anchoring sites provided by HSPGs permit initial contact between coronavirus and host cells and the concentration of virus particles on the cell surface. Coronavirus rolls onto the cell membrane by binding to HSPGs and scans for specific entry receptors (ACEII), which leads to subsequent cell entry.

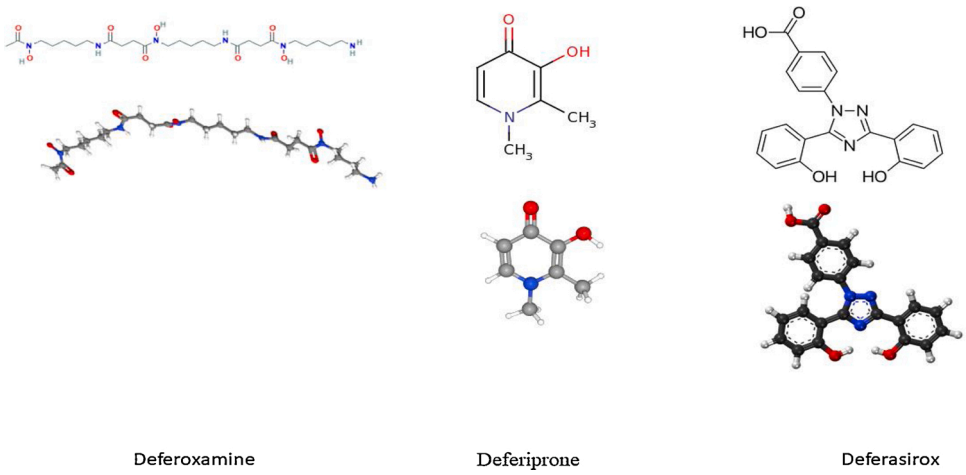

Fig. 3. Iron chelator structures.

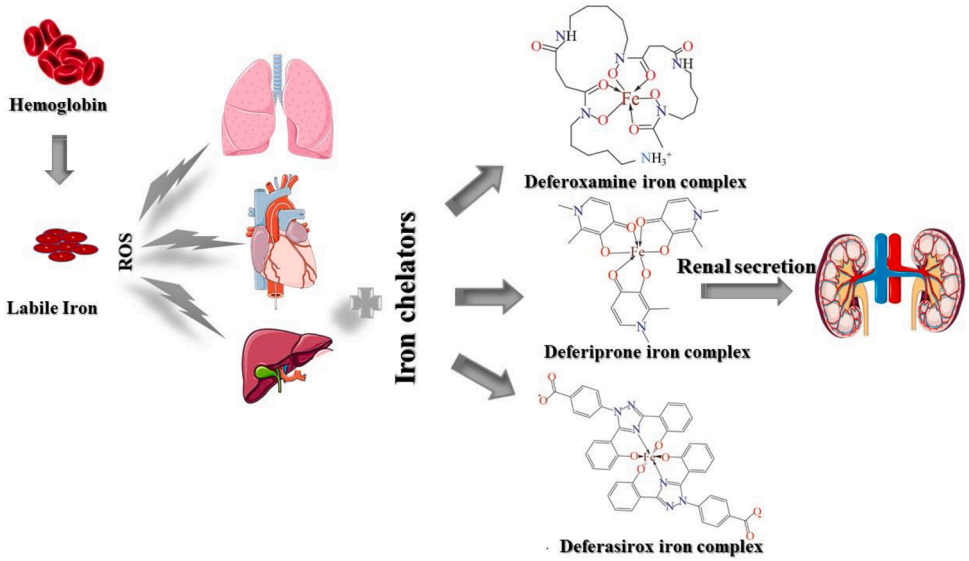

Fig. 4. Mechanism of action of iron chelators.

5.2. *Iron chelators as antivirals*

The significance of cellular iron homeostasis in viral invasion and survival is apparent through evidence displaying the reliance viral replication has on iron and the modulation of host iron metabolism by viruses [131]. The importance and relationship between the two encourage investigation into iron chelation treatment strategies for viral infections [131]. One strategy involves utilizing iron chelators' abilities to bind free iron or deplete iron from proteins containing iron [134]. Large doses of deferiprone, which has a selective affinity towards iron ions, has exhibited a prolonged survival in AIDS patients following an HIV-1 infection [131]. Additionally, increasing evidence reveals that iron chelators may possess antiviral qualities by targeting HIV-1 replication. Deferoxamine, for example, suppresses the enzymatic activity of ribonucleotide reductase 2 involved in opposite transcription which contains non-heme iron [135]. Moreover, a significant HIV-1 RNA reduction was observed in patients on deferiprone while on-drug and for up to 8-weeks off-drug without viral rebound [136]. Both deferoxamine and deferiprone exhibited inhibition of HIV-1 replication in human PBMCs and macrophages; however, this inhibition is associated with a decline in cell proliferation [137]. Similar to the two aforementioned iron chelators, the oral uptake of bidentate iron chelators, such as CP502 and CP511, has been shown to prevent HIV-1 replication through the same method of decreasing cellular proliferation [137]. Furthermore, iron chelators could impede the instigation of host cell enlargement by viruses (e.g., HCMV) through suppression of mitochondrial activity and macromolecular synthesis [138].

Drakesmith & Prentice [138] also reported that iron chelators vary in their ability to restrict aspects of the HIV-1 life cycle and in their mechanism of inhibitory action. Additionally, to secure a sufficient nucleotide supply, some large DNA viruses are known to encode their ribonucleotide reductases. HSV-1 and vaccinia virus ribonucleotide reductases are, similar to their human counterparts, iron-dependent [138]. This means that if iron availability is restricted to the viral enzyme, through the use of iron chelators, viral replication is suspended [138]. Nevertheless, iron chelators still may be scrutinized for their antiviral activity for COVID-19.

6. Conclusion

As this paper discussed, one of the most common complications that result from COVID-19 is Acute Respiratory Distress Syndrome, in which the virus replicates within the host cells and invades the surface of the lung, causing inflammation and hypercoagulation. The pulmonary

inefficiency observed in COVID-19 patients may not be caused by cell damage in the lungs alone. The virus may also attack and destroy hemoglobin, thus resulting in the release of iron from porphyrins and discharging it into the circulation with consequential iron overload. In order to compensate for the high iron level, ferritin production is boosted. The raised serum ferritin levels can cause death of hepatic cells triggering release of iron from ferritin, which results in higher levels of systemic free iron. The overabundance of free iron can exacerbate inflammatory conditions through ROS-induced oxidative damage and ferroptosis. If left untreated, ferroptosis promotes a series of reactions that further inflammation and result in multiple organ failure, pulmonary injury, and reduced lung capacity. Free iron can also contribute to the hypercoagulation found in severe COVID-19 patients through non-enzymatic conversion of fibrinogen to fibrin clots via the generation of the hydroxyl radical. The relatively lower risk for COVID-19 observed recently in individuals with blood group O compared to other blood groups may be linked to the lower serum iron status observed in these individuals.

Since iron overload contributes to COVID-19, one of the potential treatments used is lactoferrin (Lf). This glycoprotein has remained a part of the body's natural immunity due to its range of therapeutic effects. Lf has been found in the body in two forms: holo-Lf and apo-Lf; with the former referring to a compact form where iron molecules are isolated from the environment and the latter referring to iron-depleted lactoferrin (<5% saturation). The positively-charged pockets in its structure ensure maximum potential for Fe^{3+} binding and its relationship with CD4+ T-cells makes it a suitable treatment option. It causes Th1 polarization, leading to macrophage activation by connecting to surface receptors and modulating T-cell activity. Aside from the effects on the immune system, lactoferrin also reduces inflammation by modulating cytokine and ROS production, which in turn reduces iron overload. Lf has also been found to inhibit binding of heparan sulfate proteoglycans, preventing viral entry.

Another method of treating iron overload lies in the usage of iron chelators. Excessive amounts of free iron in the blood of patients with iron overload can cause cardiac iron toxicity due to a rise in ROS production. This has been treated with the chelators, including deferoxamine, deferasirox, and deferiprone; each with different properties that may determine their efficiency in relation to treating iron overload.

Contributors

HMH initiated the idea of the Review, did the literature search, and developed the first draft of the manuscript. All authors contributed to the drafting and revision of the manuscript and read and approved the final version.

Declaration of Competing Interest

All authors declare no other competing interests.

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