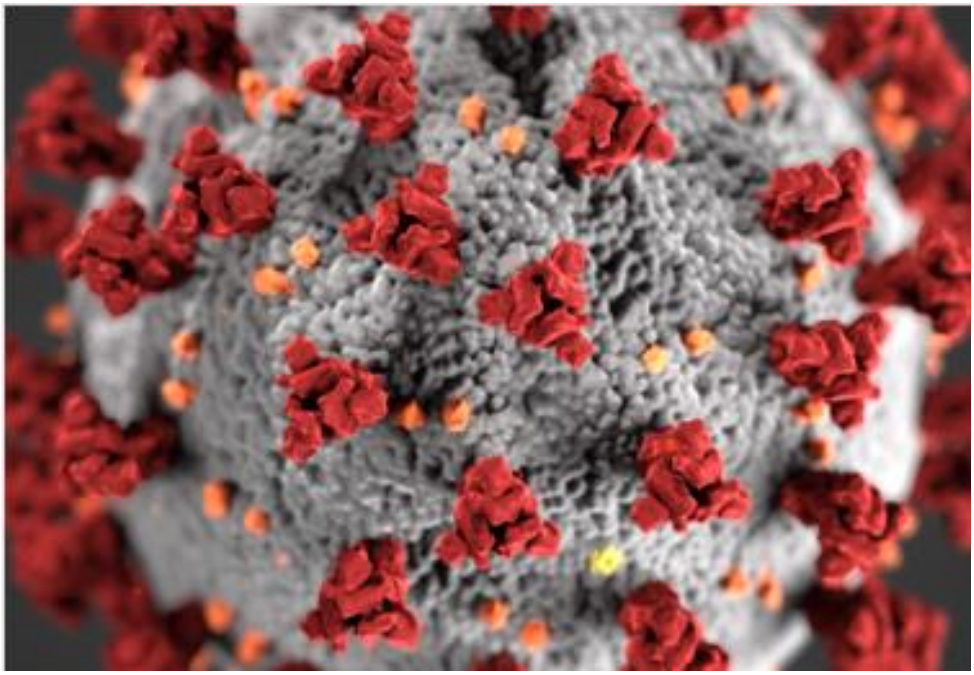
COVID-19 MUTATIONS, VACCINES & NITRIC OXIDE – THE VITAMIN C CONNECTION

BY DORIS LOH



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Doris Loh is a medical researcher and writer specializing in the investigation of familiar and innovative health topics using unique perspectives, and traditional and quantum biology.
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COVID-19 MUTATIONS, VACCINES & NITRIC OXIDE – THE VITAMIN C CONNECTION

COVID-19 is caused by a new betacoronavirus that is pathogenic and highly contagious. COVID-19 is now considered by the World Health Organization to be capable of exerting tremendous social, health and economic impacts on a global scale.

When this new virus was first identified in December, 2019, it was referred to as 2019-nCoV. '2019' to designate the year it was identified, 'n' represents 'novel', and CoV stands for coronavirus. It is considered a NOVEL virus because the genetic sequences of SARS-CoV-2, which is the actual name of the coronavirus that causes the disease COVID-19, contain only 79% similarity to SARS-CoV (2003) and 50% similarity to MERS-CoV (2012-2013), both of which are also coronaviruses [1, 2, 3].

On February 11, the novel coronavirus that first emerged in Wuhan China was officially given the name "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" and on the same day, the World Health Organization (WHO) announced COVID-19 as the name of the disease caused by SARS-CoV-2 [4, 5]. The COVID-19 virus was named SARS-CoV-**2** because of the 79% genetic similarity to the coronavirus that caused the SARS outbreak of 2003 [14].

Comparing SARS-CoV 2003 and SARS-CoV-2 Today

Severe acute respiratory syndrome (SARS) was caused by the coronavirus known as SARS-CoV, first identified in November 2002 in the Guangdong province of southern China [9]. The SARS coronavirus quickly proceeded to spread to a total of 26 countries in North and South America, Europe, and Asia before it was fully contained by the end of 2003. During the outbreak WHO reported a total of 8,098 people were infected by the SARS virus around the globe. Of those, 774 died. In the United States, only 8 people reported lab confirmed infection by SARS-CoV [2].

As of March 6 2020, SARS-CoV-2 has infected 101,858 people worldwide since its discovery at the end of 2019. Of these 101,858 patients, 3,462 have died. The SARS-CoV-2 increased more than five-fold in just one month, from 20,000 cases reported on February 3, 2020.
96 countries reported confirmed cases of COVID-19 as of March 6, 2020. There is no indication that the virus has been fully contained globally, but the rate of infection appears to have abated somewhat in China [6].
People infected by SARS in 2003 typically showed symptoms including persistent fever, chills/rigor, myalgia, malaise, dry cough, headache, and dyspnoea [7]. These clinical presentations are dramatically different from those of COVID-19.
COVID-19 patients can be asymptomatic (no symptoms) or show nonspecific symptoms. However, once symptoms emerge, they can progress quickly to cause severe pneumonia and death. Clinical features of COVID-19 patients have been seen to range from mild upper respiratory syndrome to severe bilateral pneumonia affecting both lungs. The severe viral pneumonia could cause life-threatening multiple organ failure leading to death [10].
According to a report from WHO, as of February 20, 2020, based on 55,924 confirmed laboratory cases, typical signs and symptoms of COVID-19 include "fever (87.9%), dry cough (67.7%), fatigue (38.1%), sputum production (33.4%), shortness of breath (18.6%), sore throat (13.9%), headache (13.6%), myalgia or arthralgia (14.8%), chills (11.4%), nausea or vomiting (5.0%), nasal congestion (4.8%), diarrhea (3.7%), and hemoptysis (0.9%), and conjunctival congestion (0.8%). " [8]
What is interesting is that a high risk sector has now been identified. Patients who are at the highest risk for severe disease and have higher crude fatality ratios are those over 60 years of age and have underlying conditions that include hypertension, diabetes, cardiovascular disease, chronic respiratory disease and cancer.

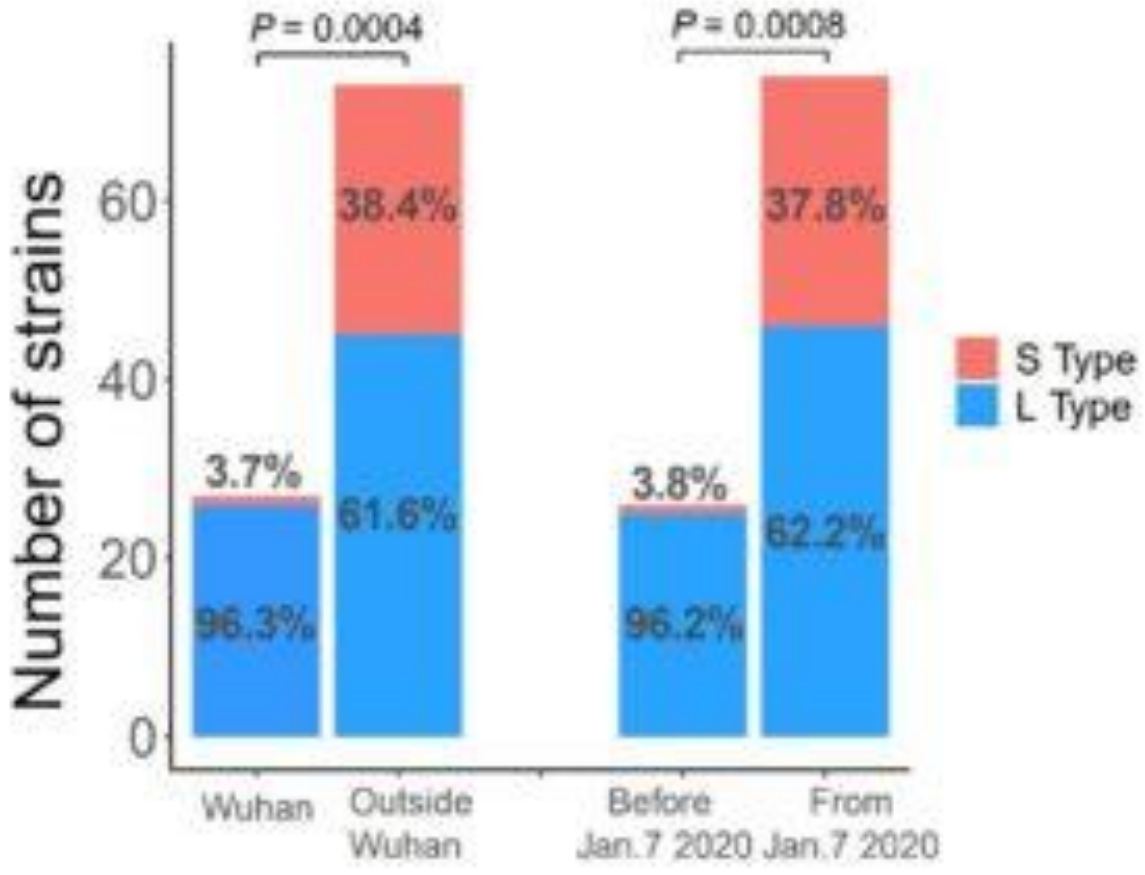
The Crude Fatality Ratio (CFR) of COVID-19 – A Story About Mutations

As of February 20, 2020, the crude fatality ratio (CFR, or Case Fatality Rate) of COVID-19 is at 3.8%, or 2,114 deaths out of 55,924 lab confirmed cases.
It is interesting to note that the CFR has **decreased significantly from 17.3% from the first ten days in January, 2020, to 0.7% for patients with symptom onset after February 1, 2020** [8]. The change in CRF rates after February 1 may reflect the active mutations of SARS-

CoV-2. According to data currently available, SARS-CoV-2 is mutating at the rate of 2 mutations per month [12].

As of March 3, 2020, there are two major haplotypes of SARS-CoV-2 consisting of the major L type with about 70% prevalence. This L haplotype had evolved from the minor S type, currently at about 30% prevalence [11]. Due to human interventions on the progression of the disease, selection pressure on the L type, which is more aggressive and highly transmissible, actually caused a reduction in frequency of this haplotype after early January 2020.

The L type frequency declined from 96.2% (before Jan 7, 2020) to about 62.2% (after January 7, 2020). In the meanwhile, the S type, a less aggressive and older virus, increased in relative frequency due to weaker selective pressure [11]. This also means that in order to continue survival and proliferation, the more aggressive L type must adapt and make changes.



[Source: Xiaolu Tang , Changcheng Wu, Xiang Li, Yuhe Song et al. : On the origin and continuing evolution of SARS-CoV-2 National Science Review, nwaa036, https://doi.org/10.1093/nsr/nwaa036 Published: 03 March 2020]

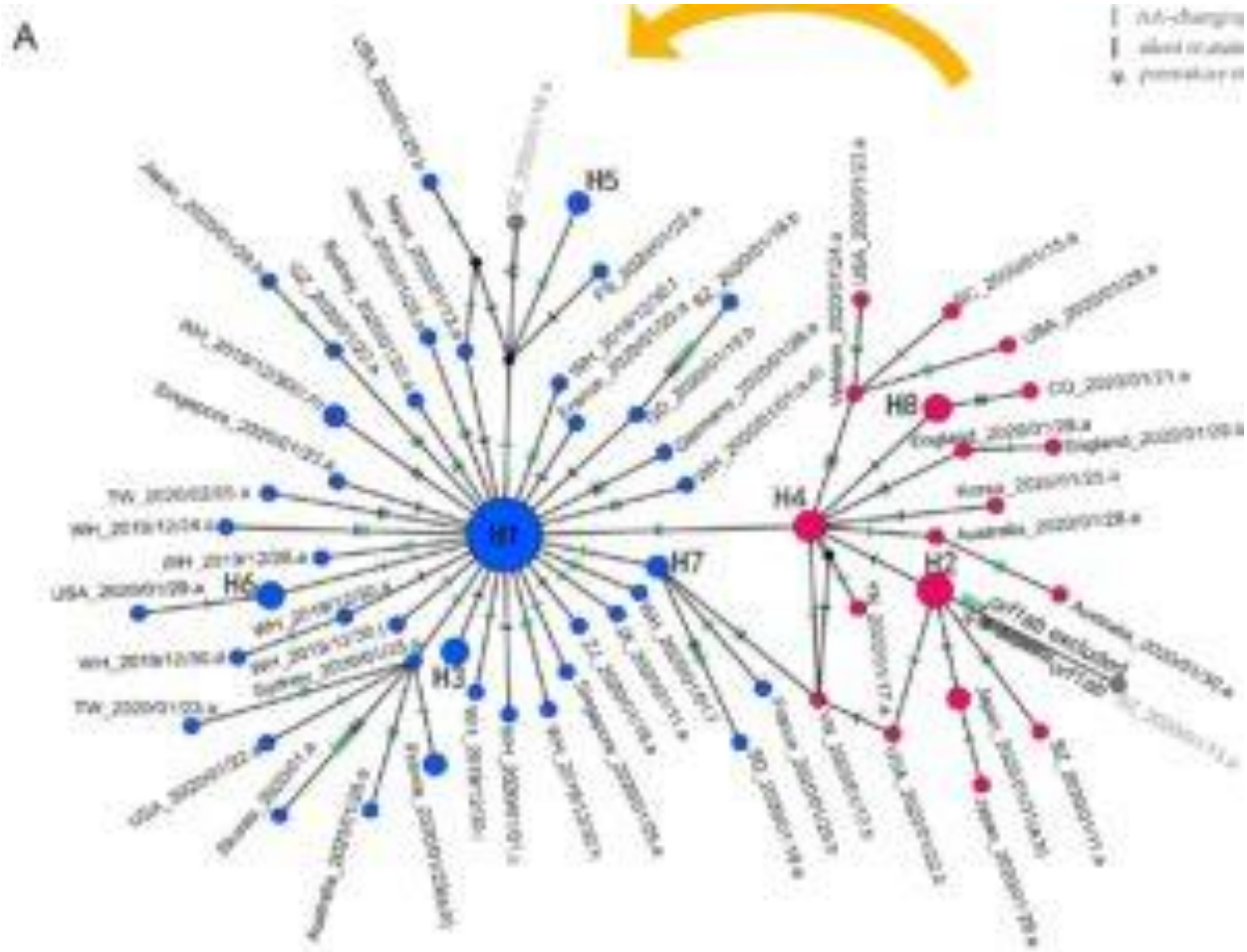
SARS Coronaviruses & Spike Protein Mutations – The Vaccine Connection

As of the publication of this article, there are no registered therapies for the treatment of coronavirus infections. No drug nor vaccine has been approved for human coronaviruses, including the SARS-CoV of 2003. How is that even possible? The key to this question lies with the spike protein in betacoronaviruses such as SARS-CoV and SARS-CoV-2.

The spike protein of SARS coronaviruses contain receptor binding sites for host receptors, and is the major facilitator for viral entry in host cells. Spike proteins will fuse viral and host cell membranes after attachment is completed to initiate infection process. Therefore the spike protein is the most critical, and the main target of neutralizing antibodies [17, 18, 19, 20, 21]. Most vaccines and antivirals target S proteins [22].

Since the spike protein is highly immunogenic because of the way it binds and infects hosts, it is therefore not surprising that this protein is under great selection pressure in the host and becomes the fastest evolving protein of the virus.

As of February 25, 2020 over 103 strains of SARS-CoV-2 have been identified. These mutations have been found in different provinces in China including Shenzhen; Shandong; Sichuan; Jiangxi; Jiangsu; Hangzhou; Guangzhou; Guangdong; Foshan; and Chongqing. Globally, mutations of SARS-CoV-2 have been collected and analyzed in countries including Australia, Belgium, England, France, Germany, Japan, Korea, Nepal, Singapore, Taiwan, USA and Vietnam. [11, 12, 13].



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Among the 103 SARS-CoV-2 virus strains, 101 of them exhibited complete linkage between the L type and the S type. 72 strains exhibited a "CT" haplotype belonging to the "L" type and 29 strains exhibited a "TC" haplotype belonging to the "S" type [11].

The identification of a furin cleavage site in the spike protein of SARS-CoV-2 not only distinguished this 2019 coronavirus from other SARS-related CoV, including the SARS-CoV of 2003, but rendered this virus to be more infectious than other coronaviruses that do not harbor furin cleavage sites in their spike proteins [23, 24, 25]. SARS-CoV-2 is estimated to be more infectious by up to 1,000 times compared to other coronaviruses as a result of the furin cleavage site mutation [26, 27].

Compared to SARS-CoV-2, the mutation rate of SARS-CoV (2003) could be considered as exceedingly moderate, with the implication that this coronavirus could actually have been co-

existing with humans as early as spring of 2002 without causing a severe epidemic [28]. If you think about this for a moment, you will realize that even with a moderate mutation rate, no effective antiviral nor vaccine had been approved for SARS-CoV since its outbreak in 2003. Why?

The receptor binding domain (RBD) of the SARS-CoV (2003) spike protein is where viral entry and neutralization is targeted by antivirals and vaccines. Scientists were able to show that **natural mutations in the RBD rendered the virus resistant to spike protein-based SARS-CoV vaccines.** As mutations evolve, the amino acid changes in RBDs on the one hand, reduced the level of binding and entry into host cells, but at the same time, also rendered the coronavirus **EXTREMELY** resistant to therapies that targeted the spike proteins [29].

The spike protein of SARS-CoV-2 is currently the rage and central focus in the development of therapeutic strategies to block the coronavirus from entering host cells [30]. Pharmaceutical giants, biotech companies and vaccine developers such as Gilead, Moderna and Novavax, as well as researchers and specialists are all racing against time to develop vaccines and antiviral drugs that target the spike protein of SARS-CoV-2 [31, 32, 24, 33, 34].

So how quickly has the SARS-CoV-2 been mutating?

Synonymous and Non-Synonymous Mutations in SARS-CoV-2 Spike Protein

Tang et al. (March 3, 2020) published a paper citing the unusually large dS value for the spike gene compared to other genes of SARS-CoV-2. This observation implied that there was a very high mutation rate in the spike protein that was coupled with a very strong natural selection process [11].

dS/dN ratios measure nucleotide substitutions in gene coding that can either be synonymous where the amino acids are not changed, or non-synonymous, where amino acids are altered [35]. This ratio is extremely effective in the determination of natural selection acting on protein-coding genes. This ratio is often used to study selection patterns of viruses, and bacteria [36].

From the results of their in depth analysis, Tang et al. found evidence that the mutations indicated a strong NEGATIVE selection on the non-synonymous sites. According to their analysis, **87.6% to 95.6% of the nonsynonymous mutations were REMOVED by negative selection** during the evolution of this novel coronavirus**.**

However, when Tang et al. compared the mutations of SARS-CoV-2 to other coronaviruses, they concluded that the extremely elevated value for synonymous mutations could actually be the result of a high mutation rate, rather than the consequence of natural selection processes which would favor synonymous substitutions that enhanced mRNA translations.
Regardless of the cause, which requires further clarification, the L type SARS-CoV-2 was shown to be more aggressive than the S type, but due to mutations, the abundance of the L type was greatly reduced in favor of the S type soon after the outbreak in January, 2020 [11].
The extremely high mutation rate of spike protein genes now presents a unique challenge in the development of therapeutic interventions that target spike proteins in SARS-CoV-2.
The fact that some infected patients show very mild or even no symptoms at all may imply that the human body has its own effective defense against COVID-19.

Nitric Oxide and the SARS Coronaviruses

After the SARS outbreak was completely contained in late 2003, scientists continued their research efforts on this coronavirus because no effective vaccine nor drug treatment could be developed for this coronavirus [16]. What these scientists discovered may surprise you.
The ubiquitous, smallest known bioactive molecule, nitric oxide is actually an extremely effective antiviral against the SARS-CoV coronavirus.

During the SARS outbreak in 2004, the use of nitric oxide in patients infected with the coronavirus was able to reverse pulmonary hypertension, improve severe hypoxia and reduced the duration of ventilatory support compared to matched control patients infected with SARS-CoV [45]
When Keyaerts et al. (2004) tested the in vitro efficacy of organic nitric oxide donor compound S-nitroso-N-acetylpenicillamine (SNAP) as therapeutic treatment for SARS-CoV, they found that SNAP was able to greatly enhance the survival rate of cells infected by SARS-CoV. At non-toxic levels of 222 μM, SNAP released nitric oxide concentration of between 30–55 μM. Cells exposed to this amount of nitric oxide showed inhibition of viral replication, and reduction of viral cytopathic effects up to 50% [37].
In the following year (2005), Åkerström et al. demonstrated that endogenous production of nitric oxide could also stop the SARS-CoV viral replication process. Before the authors infected

monkey kidney epithelial cells with SARS-CoV, they stimulated the cells with interleukin-1β (IL-1β) and human gamma interferon (IFN-γ) to induce activation of iNOS [38].
Inducible nitric oxide synthase (iNOS) is one of the three nitric oxide producing enzymes that is only expressed in activated cells such as T-cells. macrophages, neutrophils, monocytes and endothelial cells [39, 40, 41, 42, 43]. The expression of iNOS is often found to be up-regulated during infections and diseases [44].
The results obtained by the Åkerström team with iNOS induction was an **eye-opening 82% reduction in SARS-CoV viral replication rate** [38].
On March 2nd, 2020, Xijing Hospital of China sponsored a clinical trial titled "Nitric Oxide Gas Inhalation for Severe Acute Respiratory Syndrome in COVID-19", with Massachusetts General Hospital as one of the collaborators. This randomized clinical trial aims to use nitric oxide to prevent the progression of COVID-19 [45].
Even though the current SARS-CoV-2 only has a 79% genomic resemblance to SARS-CoV (2003), the lack of nitric oxide may very well exert similar detrimental effects, as nitric oxide is a critical component of our immune response defense system [44, 46]. The discovery of a furin cleavage site in the spike protein of SARS-CoV-2 [26] may render nitric oxide even more important in the therapeutic treatment of COVID-19 than SARS-CoV.
Furin enzymes are found on almost every cell surface of the human body. SARS-CoV-2 must be cleaved by furin enzymes before the virus becomes bioactive [47]. The expression of furins are enhanced under hypoxic conditions [48]. Hypoxia can also enhance the expression of nitric oxide synthases that produce nitric oxide, such as iNOS and eNOS [50, 51]. However, the behavior of this molecule changes dramatically as it reflects surrounding cellular redox conditions.

Nitric Oxide, Furins & Crude Fatality Ratio – The Hypoxia Connection

In many ways, hypoxia provides a conducive environment for SARS-CoV-2 proliferation. Hypoxia inducible factor 1α (HIF1α) is a transcription factor that can be activated under hypoxia, or inadequate oxygen supply. Expression of furin mRNA was found to be increased rapidly in oxygen-deprived cells by HIF-1, which binds to a sequence in the gene that promotes its rapid expression [49].

When a person is critically ill, oxidative stress levels are almost always significantly elevated [48, 49]. Oxidative stress can activate HIF-1a, initiating hypoxia signaling pathways [50, 51]. The activation of HIF-1a inevitably triggers the increased expression of furins, leading to cleavage and activation of COVID-19.

Once the coronavirus is activated, cellular defense immune responses are triggered. Some patients have muted responses, while others suffered severe symptoms. Currently, the observed crude fatality ratio (CFR) for infected patients over the age of 80 is a staggering **21.9%.** Males have a slightly higher CFR of 4.7% compared to 2.8% in females. What is most revealing is the CFR for the various comorbidities [8].

As of February 20, 2020 the CFR in COVID-19 for cardiovascular disease is 13.2%; diabetes is 9.2%, hypertension is 8.4%, chronic respiratory disease is 8.0%, while cancer is 7.6% [8]. The common denominators shared by all these different pathologies are all intricately related to each other.

Oxidative stress, hypoxia and nitric oxide dysregulation all play important roles in the pathology of cardiovascular diseases, diabetes, hypertension, respiratory diseases and especially cancer [70, 71, 72, 73, 74].

The deep relationship between nitric oxide synthesis and hypoxia explains why this molecule plays an essential role in COVID-19 disease progression.

Hypoxia, or the lack of adequate oxygen, can increase the production of nitric oxide through three different nitric oxide synthases (NOS), namely neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Increased nitric oxide relaxes vascular smooth muscles and therefore can balance oxygen homeostasis by facilitating increased blood flow and oxygen delivery to cells deprived of oxygen [75].

Cells replete with oxygen will proceed to deactivate and degrade HIF-1a. When there is decreased stabilization of HIF-1a, excess furin enzymes will not be produced. This environment becomes significantly less hospitable to activation and proliferation of COVID-19 coronavirus. However, when a person is critically ill, concomitant oxidative stress will inevitably cause redox imbalance [76, 77]. Even though hypoxia can increase the expression of NOS, patients still may not be able to produce enough nitric oxide because the ability of the three nitric oxide synthases to produce adequate nitric oxide is strictly dependent upon redox balance and the state of oxidative stress in the cellular environment.

REDOX, Nitric Oxide & Vitamin C – The BH4 Connection

Ascorbic acid is widely known for its antioxidant properties. The true function of this ancient molecule as a REDOX balancer is quite underappreciated [81]. The ability of ascorbic acid to donate and accept electrons in various vital processes including the production of nitric oxide [68]; regulation of hypoxia and immune responses; maintaining membrane potential; and reducing oxidative stress all highlight the importance of this molecule as an effective and integral part of therapeutic interventions that can resolve various challenges presented by SARS-CoV-2.

In the human body, the three nitric oxide synthases nNOS, eNOS and iNOS convert L-arginine to L-citrulline and nitric oxide [53, 54].

All three nitric oxide synthases require a cofactor known as tetrahydrobiopterin (BH4) [54, 55, 56]. In order for nitric oxide synthases to utilize BH4 properly in the synthesis of nitric oxide, BH4 must be maintained in a reduced state. Excess free radicals can prevent the proper recycling of BH4 and inhibit the biosynthesis of nitric oxide [57]. Therefore, during endogenous production of nitric oxide production, BH4 is the rate-limiting step. Without BH4 in the proper redox state, nitric oxide cannot be produced.

The recycling of BH4 between its various redox states is regulated by none other than the preferred REDOX balancer, ascorbic acid.

During the enzyme cycling process, BH4 donates an electron to NOS and becomes a free radical (BH3•) as a result. To recycle this free radical back into BH4, an electron is donated by ascorbate to reduce BH3• back into BH4. What is fascinating is that other antioxidants like thiols are totally INEFFECTIVE in this reduction process [62] . If there is a deficiency of ascorbate, the BH3• free radical will not be reduced back into BH4 but continue to rapidly degrade into the inactive form, BH2.

Ascorbate is the only molecule used by the body that can efficiently reduce the BH3• free radical back into BH4. Science has demonstrated that other antioxidants like thiols are totally INEFFECTIVE in this reduction requirement. If the BH3• free radical is not properly reduced back into BH4, it will quickly degrade into the inactive BH2 [58].

The formation of BH2 is actually a very dangerous development because BH2 competes with BH4 in binding to NOS, WITHOUT the production of nitric oxide. That means BH2 effectively inhibits NO production. On top of that, increased BH2 can uncouple NOS causing the enzyme to generate superoxide instead of nitric oxide. Frequently this will initiate a free radical cascade

that produces the toxic peroxynitrite, that end up consuming nitric oxide and oxidizing more BH4 [59, 60, 61].

Åkerström et al. has conclusively shown that the induction of iNOS can reduce SARS-CoV viral replication by an eye-opening 82% [38}. It is thus not surprising at all to find that ascorbate could enhance iNOS activity by increasing tetrahydrobiopterin (BH4) concentration in vitro [63, 69].

The Role of Ascorbic Acid in Therapeutic Treatment for COVID-19

Deficiency in vitamin C not only reduces production of nitric oxide in COVID-19 patients, but can potentially exacerbate already out-of-control oxidative stress and hypoxia. Increased oxidative stress triggers the activation of the hypoxia signaling pathways that can result in increased expression of furin enzymes. Increased furin enzymes will activate more SARS-CoV-2 coronaviruses.

It is now extremely clear why the extensive use of vitamin C in China as therapeutic treatment for COVID-19 has produced most encouraging results, showing quick recovery of COVID-19 patients [67]. Ascorbic acid can act on multiple levels, reducing oxidative stress, regulating hypoxia signaling, mitochondrial membrane potential, furin expression, and modulation of immune defenses to stem the progression of cytokine storms [78, 79, 80].

However, the therapeutic manipulation of the nitric oxide molecule does come with challenges that are reinforced by compromised redox environments. Nitric oxide can be cytoprotective or cytotoxic, depending on the cellular redox context. Whether nitric oxide is used as a signaling molecule or causes inhibition of ATP production and increased ROS production leading to cell death, is completely dependent upon the redox states of intracellular enzymes that may be affected by ascorbic acid [65, 66].

A prime example would be the main safety issue highlighted in the recently proposed clinical trial for inhaled nitric oxide, where the increase of methemoglobin in blood could result from prolonged exposure to inhaled NO [45].

An increase of methemoglobin is dangerous because this form of hemoglobin is unable to bind oxygen as the iron in the heme is in the oxidized ferric form. Increased methemoglobin means that less oxygen will be delivered to cells and tissues [64]. When that happens, the use of inhaled NO can actually create more problems by increasing hypoxia. However, having adequate ascorbic acid can easily prevent an excess of methemoglobin.

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