

Review

COVID-19 pathophysiology: interactions of gut microbiome, melatonin, vitamin D, stress, kynurenine and the alpha 7 nicotinic receptor: Treatment implications

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Running Title: COVID-19 pathophysiology and Treatment

Received: May 11, 2020; Accepted: June 06, 2020

ABSTRACT

As data emerges on the pathophysiological underpinnings of severe acute respiratory syndrome coronavirus (SARS-CoV)-2, it is clear that there are considerable variations in its susceptibility and severity/fatality, which give indications as to its pathophysiology and treatment. SARS-CoV-2 modulatory factors include age, vitamin D levels, cigarette smoking, gender and ethnicity as well as premorbid medical conditions, including diabetes, cancer, obesity, cardiovascular disease, and immune-compromised conditions. A complex picture is emerging, with an array of systemic physiological processes interacting including circadian, immune, intestinal, CNS and coagulation factors. This article reviews data on SARS-CoV-2 pathoetiology and pathophysiology. It is proposed that a decrease in pineal and systemic melatonin is an important driver of SARS-CoV-2 susceptibility and severity, with the loss of pineal melatonin's induction of the alpha 7 nicotinic acetylcholine receptor ($\alpha 7nAChR$) in pulmonary epithelial cells and immune cells being a powerful regulator of susceptibility and severity, respectively. Stress, including discrimination stress, and decreased vitamin D also regulate SARS-CoV-2, including via gut dysbiosis and permeability, with a resultant decrease in the short-chain fatty acid, butyrate, and increase in circulating lipopolysaccharide. Stress and cytokine induction of the kynurenine pathways, leads to aryl hydrocarbon receptor activation, which primes platelets for heightened activity, coagulation and thrombin production, thereby driving elevations in thrombin that underpin many SARS-CoV-2 fatalities. On the basis of these pathophysiological changes, prophylactic and symptomatic treatments are proposed, including the use of melatonin and $\alpha 7nAChR$ agonism.

Key words: COVID-19, melatonin, circadian, gut, butyrate, virus, stress, kynurenine, platelets, alpha 7 nicotinic receptor.

1. INTRODUCTION

Data on the pathophysiological underpinnings of severe acute respiratory syndrome coronavirus (SARS-CoV-2) are emerging, with relevance to variations in susceptibility and severity/fatality. Variations in age, gender, skin pigmentation, vitamin D, and cigarette

smoking as well as premorbid medical conditions, such as diabetes, obesity, cardiovascular disorders (CVD), cancer and immune-compromised conditions are all associated with modulating the susceptibility and/or severity of SARS-CoV-2 infection within the COVID-19 pandemic. The current article reviews this data and proposes significant roles for variations in circadian and mitochondrial melatonin, the alpha 7 nicotinic acetylcholine receptor ($\alpha 7nAChR$), vitamin D, gut dysbiosis/permeability, platelet activation, the aryl hydrocarbon receptor (AhR), stress and the autonomic nervous system, as well as an increased conversion of tryptophan to kynurenine pathway products in providing the biological underpinnings to SARS-CoV-2 infection. This provides a framework for understanding the variations in individual susceptibility and severity/fatality, as well as cheap and readily available prophylactic and treatment interventions.

The role of various factors in SARS-CoV-2 infection are first discussed before being integrated into a wider model of the entry/susceptibility and clinical course/treatment. First, the role of stress in viral infections are reviewed.

2. STRESS AND VIRAL INFECTIONS

Psychological stress has been long-recognized to modulate susceptibility, severity and recurrence of viral infections (1,2), although the mechanisms involved have still to be ascertained. Recent work indicates that psychological stress may have its biological underpinnings driven by increased synthesis and release of corticotropin-releasing hormone (CRH) from the hypothalamus and amygdala (3). CRH can act independently of hypothalamic-pituitary-adrenal (HPA) axis activation, including via its activation of mucosal mast cells, thereby leading to upregulation of the pro-inflammatory cytokine, tumor necrosis factor (TNF)- α . TNF- α acts on gut epithelial cells to slacken tight junctions, there leading to gut permeability. An increase in gut permeability and associated dysregulation of the gut microbiome (dysbiosis) underpins how stress may modulate such a diverse array of medical conditions, with the same medical conditions also being associated with regulation by gut dysbiosis/permeability (4). The gut is therefore an important conduit for how stress, and the diverse array of stress-associated medical conditions, may modulate the susceptibility to, and pathophysiology of, SARS-CoV-2 infection within the COVID-19 pandemic (5).

3. GUT PERMEABILITY AND DYSBIOSIS

There is a growing appreciation of the importance of gut dysbiosis/permeability in a host of diverse medical conditions, including arthritis, neuro-immune disorders, and cancers, as well as an array of different psychiatric conditions (4). Two significant changes in the gut underpin the impact of the gut on such diverse medical presentations, *viz* gut dysbiosis and gut permeability. Gut dysbiosis is invariably associated with suppressed production of the gut bacteria-derived short-chain fatty acid, butyrate, whilst gut permeability allows the transfer of lipopolysaccharide (LPS), a constituent of the outer membrane of gram-negative bacteria, into the general circulation, thereby triggering immune and glia inflammatory activity. Many factors, including diet (alcohol, sugar and fat), air pollutants and epigenetic changes as well as stress, can drive gut dysbiosis/permeability (6), and thereby impact on immune and central inflammatory activity associated with SARS-CoV-2 and the COVID-19 pandemic (5).

By effects in intestinal epithelial cells, butyrate seals the gut (7). However, butyrate can also be transferred across intestinal epithelial cells in the general circulation, where it has a number of effects, including dampening systemic immunity and CNS glia activity. Butyrate is also a histone deacetylase (HDAC) inhibitor and therefore a powerful epigenetic regulator, whilst its induction of the melatonergic pathway allows it to have mitochondria-optimizing

effects. Butyrate can also increase the cytotoxicity of natural killer cells, which are the cells that the body uses to deal with cancers and viruses. It is by such effects that butyrate has impacts that are relevant to the susceptibility to, and course of, SARS-CoV-2 infection. Most of the medical conditions associated with a high risk of fatality from COVID-19 infection show evidence of gut dysbiosis, including, diabetes, obesity, cardiovascular diseases (CVD) and pulmonary conditions (4). This would suggest that the increased gut dysbiosis evident in these conditions may contribute to the initial SARS-CoV-2 infection, whilst the increase in pro-inflammatory cytokines that are part of the initial viral 'cytokine storm', will contribute to increased gut dysbiosis and permeability in the course of SARS-CoV-2 infection.

The loss of butyrate's maintenance of the gut barrier, contributes to the gut permeability that can arise from diet, stress and raised levels of circulating pro-inflammatory cytokines. The loosening of intestinal epithelial cell tight junctions allows LPS to activate toll-like receptor (TLR)4, which may also be contributed to by the concurrent release of high-mobility group box (HMGB)1 in the exosomes of intestinal epithelial cells (8). LPS and alarmins, such as HMGB1, drive TLR4 activation in immune and glial cells thereby contributing to inflammation in the pathoetiology and/or pathophysiology of an array of medical conditions. Virus-bacteria interactions are common in the course of viral infections, especially as points of entry for viruses are at mucosal surfaces that are already colonized with bacterial microbials (9). Variations in bacterial levels are of clinical importance, as shown by LPS activation of TLR4 increasing influenza fatalities (10).

There is a growing body of data showing darker skin pigmentation to be a risk factor for COVID-19 susceptibility, severity and fatality (11). Many factors are thought to contribute to this, including social crowding and a decrease in vitamin D's regulation of immunity and gut dysbiosis/permeability (12). However, the consequences of discrimination stress, including on gut dysbiosis/permeability are also likely to be relevant, including in interaction with lower vitamin D levels. Vitamin D positively regulates pulmonary epithelial cells, including anti-viral responses (13). Decreased vitamin D is undoubtedly relevant to COVID-19 symptom severity/fatality (14). However, its interaction with experiences of discrimination stress seem likely, and will be important for future research to determine.

3.1. Gut permeability/dysbiosis and SARS-CoV-2.

As a HDAC inhibitor, butyrate, including as a nutraceutical, sodium butyrate, is a powerful epigenetic regulator, which may be important to SARS-CoV-2 regulation (5). Although a number of researchers have proposed a role for epigenetic processes in the susceptibility to, and severity of, SARS-CoV-2 infection (15-6), data to date are sparse. However, HDAC inhibition regulates many viruses, including attenuating influenza-driven pneumonia infections (17). This would indicate that variations in gut microbiome-derived butyrate would modulate the likelihood and course of SARS-CoV-2 infection across all age groups and conditions. As noted, butyrate induces the melatonergic pathway, as shown in intestinal epithelial cells (7). This may be of some importance as melatonin, both pineal and within the mitochondria of all immune cells, has been proposed to modulate the susceptibility and severity of SARS-CoV-2, as well as being important to its treatment, including as a prophylactic (5). The immune-dampening effects of butyrate may be at least partly driven by its upregulation of the melatonergic pathway, including within immune cells (18), allowing the autocrine effects of melatonin to switch activated immune cells to a more quiescent state (19).

4. SARS-CoV-2 AND CIRCADIAN RHYTHM

Pro-inflammatory cytokines and stress, partly via gut dysbiosis/permeability will suppress levels of serotonin and melatonin (4). This is partly mediated by pro-inflammatory cytokine induced indoleamine 2,3-dioxygenase (IDO), leading to the driving of tryptophan away from serotonin and melatonin synthesis, to the production of kynurenine pathway products, which is covered in more detail below. An increase LPS, arising from gut permeability, may also act to suppress pineal melatonin synthesis. This is of some importance, as night-time pineal melatonin is the circadian driver of night-time immune cell dampening, whereby reactive immune and glia cells are shifted to a quiescent phenotype. The effects of melatonin are mediated via an increase in the circadian gene, *Bmal1*, leading to pyruvate dehydrogenase kinase inhibition, thereby leading to the disinhibition of the pyruvate dehydrogenase complex (PDC). PDC disinhibition drives the conversion of pyruvate to acetyl-CoA, thereby increasing oxidative phosphorylation (OXPHOS), and tricarboxylic acid (TCA) cycle ATP, with acetyl-CoA also being a necessary co-substrate for aralkylamine N-acetyltransferase (AANAT), and therefore the mitochondrial melatonergic pathway. Such changes in mitochondrial metabolism are crucial to the shifting of immune cells from a reactive to a quiescent phenotype. Consequently, factors that act to regulate pineal and/or mitochondrial melatonin, such as butyrate, LPS, stress and pro-inflammatory cytokines, will significantly modulate the immune response. Clearly, such variations in melatonin regulation are important to the pathophysiological changes occurring in response to SARS-CoV-2 and other viral infections (4).

Although different viral infections have been shown to have their susceptibility and severity significantly regulated by the circadian rhythm (20), the role of the circadian rhythm in SARS-CoV-2 infection has not been investigated to date. However, the relevance of the circadian rhythm in the pathoetiology and pathophysiology of SARS-CoV-2 has been proposed (5, 21). This would seem to be how viruses have adapted to the initial 'cytokine storm', as the rise in pro-inflammatory cytokines acts to switch off pineal melatonin production (19), and shift the host's biology to favour viral survival and proliferation. Ageing, stress, gut dysbiosis/permeability and most of the high risk conditions for host fatality in the COVID-19 pandemic are associated with a decrease in melatonin. Interestingly, melatonin shows clinical utility in these SARS-CoV-2 high risk pre-existent medical conditions, reviewed in (5). Overall, pre-existent or viral-suppressed pineal melatonin production will contribute to SARS-CoV-2 pathophysiology.

Melatonin has a number of effects relevant to SARS-CoV-2 infection, including in its capacity as an antioxidant, anti-inflammatory and optimizer of mitochondrial function. Melatonin has been selected over the course of evolution to be the body's natural regulator of the immune response, especially the night-time dampening of immune cells. A decrease in melatonin in the high risk medical conditions associated with SARS-CoV-2 severity, strongly indicates a role for variations in melatonin in the modulation of COVID-19 pandemic outcomes. The role of melatonin in regulating mitochondrial metabolism, especially in immune cells, may be of particular importance. The potential role of melatonin in SARS-CoV-2 has been reviewed previously (5). One important aspect of pineal melatonin effects is the induction of the alpha 7 nicotinic acetylcholine receptor ($\alpha 7nAChR$) (22). The role of the $\alpha 7nAChR$ in SARS-CoV-2 infection is reviewed next.

5. ALPHA 7 NICOTINIC RECEPTOR AND COVID-19 RISK

The $\alpha 7nAChR$ is an important mediator of many of pineal melatonin's effects (19, 23). Given its immune dampening and mitochondria-optimizing effects, $\alpha 7nAChR$ agonists have

recently been proposed to have 'cognitive enhancing' effects across a number of medical conditions, including Alzheimer's disease and schizophrenia (23). However, the $\alpha 7$ nAChR is also expressed in lung epithelial cells within lipid rafts, as is the angiotensin converting enzyme (ACE)2 receptor. The ACE2 receptor is where the SARS-CoV-2 virus gains entry into lung epithelial cells. As to whether the melatonin-regulated levels of $\alpha 7$ nAChR in lung epithelial cells modulate ACE2 receptor, thereby regulating viral entry requires investigation. Under conditions of severe LPS infection in the lung, $\alpha 7$ nAChR activation provides significant benefit, partly via the regulation of autophagy (24). As such, factors that act to inhibit pineal, and perhaps local epithelial cell, melatonin production, via decreased induction of the $\alpha 7$ nAChR in lung epithelial cells, may modulate the levels of SARS-CoV-2 entry into the lung and thereby infection susceptibility as well as levels of severity and fatality.

This may be parsimonious with the data from France showing cigarette smokers to have a decreased risk of COVID-19 infection (25), which has led to a wide utilization of nicotine gum and patches in the hope of decreasing infection risk. Nicotine increases $\alpha 7$ nAChR levels and activation, suggesting that using nicotine products may well have utility in modulating SARS-CoV-2 entry and infection severity. As well as effects in pulmonary epithelial cells, the $\alpha 7$ nAChR acts to dampen immune cell activity (26), suggesting that some of the night-time dampening effects of melatonin on immune cell reactivity may be mediated, at least partly, via the $\alpha 7$ nAChR. As such, agonists at the $\alpha 7$ nAChR, including cigarette-derived nicotine can not only have stress-alleviating effects, but direct effects on immune cell reactivity. Unfortunately, for cigarette smokers, the many other components of cigarette smoke have adverse health consequences that contribute to an increase in COVID-19 severity and fatality, once an infection does occur.

It should also be noted that the increase in fatalities arising from COVID-19 in the elderly may be contributed to by the raised levels of circulating β -amyloid ($A\beta$), both centrally and systemically. An increase in $A\beta$ is classically associated with Alzheimer's disease, with effects that traditionally have been attributed to the detrimental effects of $A\beta$ plaques on neuronal function and survival. However, there is a growing appreciation that some of the effects of the most toxic form of $A\beta$, $A\beta 42$, arise in the very early prodromal stages of dementia, when $A\beta 42$ activates pinealocyte and/or pineal microglia TLR4, which suppresses pineal melatonin production (27). Consequently, the raised levels of $A\beta 42$ over ageing, especially in prodromal dementia, is accompanied by the loss of pineal melatonin's regulation of the circadian rhythm, including its night-time dampening of immune cell activation. As noted, such dampening of immune cells may be partly mediated by melatonin's induction of the $\alpha 7$ nAChR. This is further complicated by $A\beta 42$ binding to, and suppression of, the $\alpha 7$ nAChR. As such, the raised levels of $A\beta 42$ in the elderly will have consequences for both melatonin and $\alpha 7$ nAChR levels and effects, including on central and systemic immune cell regulation. Such changes are likely to modulate both the susceptibility to, and severity of, COVID-19 infection.

As highlighted above, the effects of psychological stress in the modulation of COVID-19 severity may be mediated by an increase in gut permeability/dysbiosis. Preclinical data shows that the beneficial effects of melatonin on gut permeability are via $\alpha 7$ nAChR activation (28), which the authors propose is mediated by melatonin leading to an increase in vagal nerve ACh release, which then activates the $\alpha 7$ nAChR on gut epithelial cells and/or mucosal immune cells. As such, melatonin and the $\alpha 7$ nAChR may be intimately linked to the regulation of gut permeability/dysbiosis and the role that this has in COVID-19 infections. Although the $\alpha 7$ nAChR can be epigenetically regulated (29), it is unknown as to whether butyrate's HDAC inhibition modulates $\alpha 7$ nAChR levels or function. This will be important to determine, as it could suggest that gut dysbiosis will impact on the immune and pulmonary regulatory effects of the $\alpha 7$ nAChR, with relevance to SARS-CoV-2 entry, susceptibility and

severity. Butyrate's induction of the melatonergic pathway, as shown in intestinal epithelial cells (7), may also be relevant to $\alpha 7$ nAChR regulation and requires investigation.

5.1. dup $\alpha 7$ nAChR.

The powerful and strongly positive effects of $\alpha 7$ nAChR agonists on cognition and wider health in preclinical models has not been realized in human studies. Although showing some significant beneficial effects, $\alpha 7$ nAChR agonists have been relatively disappointing. This seems as a consequence of the uniquely human duplicate, dup $\alpha 7$ (CHRFAM7A), which negatively regulates the $\alpha 7$ nAChR. As the $\alpha 7$ nAChR and dup $\alpha 7$ are differentially regulated, both genetically and epigenetically, the seemingly clear beneficial effects of $\alpha 7$ nAChR agonism are less predictable in the absence of concurrent measures of dup $\alpha 7$ levels and activity. Consequently, the effects of melatonin, via the $\alpha 7$ nAChR, are complicated by factors acting to regulate the levels and effects of dup $\alpha 7$. The interactions of $\alpha 7$ nAChR and dup $\alpha 7$ in immune cells, gut and pulmonary epithelial cells will be important to determine, including in relation to SARS-CoV-2 and the COVID-19 pandemic, but also more widely over the course of development (30).

It should also be noted that dup $\alpha 7$ /CHRFAM7A single nucleotide polymorphisms (SNP) can dramatically increase the immune-inflammatory response in human medical conditions (31), highlighting the importance of variations in dup $\alpha 7$ alleles, levels and activity in the regulation of inflammatory responses. Expression levels of dup $\alpha 7$ /CHRFAM7A are high in circulating blood cells, suggesting important systemic impacts (31). Importantly, dup $\alpha 7$ /CHRFAM7A has been shown to have regulatory effects in both pulmonary (32) and intestinal epithelial cells (33), indicating that it will have a relevant impact on the proposed protection afforded by the $\alpha 7$ nAChR at both of these sites, including in regard to SARS-CoV-2 infection susceptibility and severity. This is further supported by the data showing that LPS acts to regulate dup $\alpha 7$ /CHRFAM7A transcription (33-4), suggesting that an increase in gut permeability and the detrimental effects of bacterial infection on viral infection severity, may be regulated via variations in dup $\alpha 7$ /CHRFAM7A level modulating $\alpha 7$ nAChR function. As a dominant negative inhibitor of the $\alpha 7$ nAChR/CHRNA7 (35), dup $\alpha 7$ /CHRFAM7A alters agonist-induced signalling as well as negatively modulating $\alpha 7$ nAChR/CHRNA7 gene expression, assembly and functional effects on cells (36). The natural ligand for dup $\alpha 7$ /CHRFAM7A has still to be identified. The dup $\alpha 7$ /CHRFAM7A can form plasma membrane hetero-polymers with $\alpha 7$ nAChR, as well as regulate its expression at the plasma membrane. It has been proposed that the dup $\alpha 7$ /CHRFAM7A in intestinal epithelial cells may even afford protection of these cells against the high levels of LPS to which they are exposed (33).

The gene delivery of dup $\alpha 7$ /CHRFAM7A into the human monocytic cell line, THP-1, attenuates cell migration and chemotaxis to monocyte chemoattractant protein, and lowers colony formation, indicating that dup $\alpha 7$ /CHRFAM7A can modulate the biological activity and migration of monocytes/macrophages (37). The transgenic expression of dup $\alpha 7$ /CHRFAM7A alters the murine immune response in a model of systemic inflammatory response syndrome (SIRS), with the transgenic expression of dup $\alpha 7$ /CHRFAM7A amplifying the murine inflammatory response (38). These authors also showed that dup $\alpha 7$ /CHRFAM7A is expressed in human leukocytes, where it enhances cell-cell adhesion, as well as modulating gene expressions associated with the migration of leukocytes. Clearly, dup $\alpha 7$ /CHRFAM7A has the capacity to significantly regulate the human immune response, as well as responses within epithelial cells. The dup $\alpha 7$ /CHRFAM7A impact on $\alpha 7$ nAChR presence at the plasma membrane is likely to regulate the nature of the complexes formed in lipid rafts in these cells, including possibly the expression, or fine-tuned structural conformation, of the ACE2

receptor.

The levels and interactions of the ACE2 receptor, dup α 7 and α 7nAChR in the lipid rafts of human pulmonary epithelial cells and how their interactions modulate the impact of the SARS-CoV-2 will be important to determine. As the presence of the ACE2 receptor in lipid rafts is required for the original SARS-CoV entry into pulmonary epithelial cells (39), which seems the same for SARS-CoV-2 (40), clearly the presence of, and perhaps organizational variations in, lipid rafts, will modulate SARS-CoV-2 entry and therefore SARS-CoV-2 infection susceptibility. If dup α 7/CHRFAM7A is relevant to SARS-CoV-2 entry, it is not unlikely that this will be co-ordinated with an altered pulmonary epithelial cell and immune response.

As to whether dup α 7/CHRFAM7A is regulated over the circadian rhythm, like the α 7nAChR, is unknown, nor is it known if melatonin regulates dup α 7/CHRFAM7A levels. This will be important to determine. Clearly, the upregulation of the α 7nAChR by melatonin over the circadian rhythm could have its immune and cellular regulatory impacts negated by a concurrent increase in dup α 7/CHRFAM7A. It is also important to note that there are considerable variations in the dup α 7/ α 7nAChR in different cell types, with this ratio being 1000:1 in macrophages, but 1:3 in the SH-SY5Y neuronal cells and human brain cells (41). As to how this ratio varies over the circadian rhythm, as well as by ageing, smoking and mitochondrial function, in pulmonary epithelial cells and immune cells will be important to determine.

Interestingly, human immunodeficiency virus type 1 (HIV-1)-induced neurocognitive disorders (HAND), mediated by the HIV-1 envelope protein gp120IIIB, markedly increases the α 7nAChR in neurons, which paradoxical to most α 7nAChR data, contributes to neuronal death (42). The effects of gp120IIIB also include a suppression of dup α 7/CHRFAM7A in neuronal cells, which is proposed by the authors to contribute to neuronal loss and cognitive disorders. As to whether the SARS-CoV-2 virus acts to regulate the levels and ratio of dup α 7 and α 7nAChR in different cell types will be important to determine.

The relatively little data on dup α 7/CHRFAM7A regulation considerably complicates the role of the α 7nAChR in SARS-CoV-2 entry and effects. A simple treatment model of melatonin administration leading to increased α 7nAChR levels that protect gut and lung, whilst acting to regulate immune responses and possibly SARS-CoV-2 entry via the ACE2 receptor on pulmonary epithelial cells lipid rafts, may be considerably complicated by the presence of dup α 7/CHRFAM7A. Clearly, dup α 7/CHRFAM7A SNPs, levels and interactions with SARS-CoV-2 survival and symptom severity will be important to determine. Of particular importance, will be research as the effects of circadian melatonin on dup α 7/CHRFAM7A levels in different cell types. The effects of melatonin in upregulating α 7nAChR levels and effects (19) would strongly suggest that there is no concurrent upregulation of dup α 7/CHRFAM7A. Factors acting to regulate dup α 7/CHRFAM7A in different cell types will be important to determine, including as to whether any SARS-CoV-2 proteins, like HIV-1's gp120IIIB, are relevant to this. Given that the α 7nAChR is also present on the mitochondria membrane, it requires clarification as to whether dup α 7/CHRFAM7A binds with mitochondrial α 7nAChR to regulate mitochondrial Ca²⁺ influx.

In the absence of dup α 7/CHRFAM7A data, it is assumed that melatonin's induction of α 7nAChR will positively modulate epithelial and immune responses to SARS-CoV-2, with some α 7nAChR-independent effects (5).

6. PLATELETS, EMBOLISMS AND α 7nAChR

Alterations in baseline platelet count are often evident in SARS-CoV-2 patients at time of hospital admission, with a low platelet count (thrombocytopenia), a common consequence of

viral infection 43), being associated with a three-fold increase in fatality risk (44). Lower platelet counts correlate with raised levels of fibrin(ogen), with both being associated with infection severity (45), being coupled to relatively raised neutrophil levels and lower lymphocyte levels (46). However, higher platelet peaks over the course of admission are also associated with more severe infection and increased mortality, which is proposed to arise in correlation with heightened levels of pro-inflammatory cytokines during the 'cytokine storm' (47). Thrombin time, the time to thrombin-induced clotting, is also decreased in SARS-CoV-2 infected patients, coupled to raised levels of D-dimer and other fibrin(ogen) degradation products (48), indicating significant alterations in coagulation regulation. The emerging consensus indicates SARS-CoV-2 fatalities are intimately linked to prothrombotic disseminated intravascular coagulation, with raised fibrin deposition in pulmonary vasculature coupled to high rates of vascular occlusive events, including stroke (49). New stroke presentations, including in the young, may be associated with an increased likelihood of SARS-Cov-2 infection (50).

Such changes would predict an increase in central demyelination in COVID-19 patients, given the role of platelet activation in driving elevations in thrombin and fibrin that underpin the demyelination and lower remyelination evident in many CNS conditions, including multiple sclerosis (51). This suggests that alterations in platelet-modulated coagulation will contribute to the central changes that can be evident in SARS-CoV-2, which is supported by data showing severe SARS-CoV-2 symptoms to be associated with brain and spinal demyelinating lesions (52). Although central changes in SARS-CoV-2 patients may be mediated by the virus gaining entry to the brain via the vasculature or peripheral nerves (53), alterations in coagulation processes may also be relevant, including via blood-brain barrier (BBB) breakdown contributing to viral entry into the CNS.

Platelet activation and an increase in coagulation factors are more prevalent in the high-risk conditions associated with SARS-CoV-2 fatalities, including type II diabetes and obesity (54), with platelet activation being driven by heightened levels of reactive oxygen species (ROS) (55). Platelet activation is classically associated with an increase in peroxynitrite, which induces acidic sphingomyelinase (aSMase) and ceramide. aSMase induction is a major driver of platelet activation and thrombin formation (56). Trimethylamine N-oxide (TMAO) is another factor commonly increased factor in obesity and type II diabetes, which increases platelet activation. The decrease in melatonin and butyrate that are common in obesity/diabetes will contribute to platelet activation, reviewed in (51).

The central changes occurring in SARS-CoV-2 patients may be intimately linked to wider systemic processes. Gut dysbiosis/permeability, via suppressed butyrate and increased LPS, contribute to a wide array of pathophysiological processes that are evident in SARS-CoV-2 patients, including heightened immune-inflammation, oxidative stress, circulating LPS, and platelet activation. Centrally, these factors act via microglia and astrocyte ceramide induction, which underpins demyelination and the suppression of remyelination, coupled to an increase in BBB permeability. The two-way interactions of obesity/lipid dysregulation with gut dysbiosis/permeability, via increased TMAO and decreased butyrate, contribute to platelet activation and thereby to an increase in central thrombin and fibrin that heighten the risk of stroke, embolisms and myocardial infarction.

Emerging data indicates that a significant proportion of fatalities from SARS-CoV-2 arise from thrombo-embolisms (57). As to how this interacts with variations in platelet counts/activation as well as thrombin and fibrin(ogen) levels awaits clarification. Human platelets express the α_7nAChR , the activation of which may modulate platelet function (58). There is no data re dup α_7 /CHRFAM7A in platelets, and therefore it is unknown if dup α_7 acts to prevent α_7nAChR activation effects in platelets. Some of the detrimental effects of cigarette smoking are mediated via non-nicotine dependent platelet activation, especially via

the cigarette smoke component, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which primes platelets for increased activation and aggregation (59). This would also suggest that the endogenous ligands of the AhR, such as 6-Formylindolo[3,2-b]Carbazole (FICZ), and/or pro-inflammatory cytokine induced kynurenine, may be contributing to AhR-induced platelet activation and aggregation. It is highly likely that the raised levels of pro-inflammatory cytokines in SARS-CoV-2 infection will activate IDO to increase the kynurenine pathway products, such as kynurenine and kynurenic acid, which can activate the AhR, including on platelets. As such, cigarette smoking, pro-inflammatory cytokines and stress can modulate platelet activation and aggregation, and therefore heightened levels of thrombin, fibrin and thrombo-embolisms that significantly contribute to SARS-CoV-2 fatalities. It may be important to note that poor clinical outcomes in influenza patients are associated with an increase in IDO and the activation of the kynurenine pathway (60).

7. PRO-INFLAMMATORY CYTOKINES AND KYNURENINE

As in other infections, the raised levels of pro-inflammatory cytokines in SARS-CoV-2 patients will raise IDO levels, leading to the driving of tryptophan away from serotonin, N-acetylserotonin (NAS) and melatonin synthesis, by driving tryptophan to down the kynurenine pathway. This is common in the wide array of diverse medical conditions associated with raised levels of pro-inflammatory cytokines, including depression, Alzheimer's disease, multiple sclerosis, cancers and CVDs (61-64). It is such processes that underpin the high levels of depression that are associated with these conditions as a consequence of the decreased serotonin, NAS and melatonin and heightened kynurenine pathway activation that ensues (65). As such, depression is not a 'comorbidity' of these diverse conditions, but, at least in part, arises from the overlapping biological underpinnings, viz cytokine-induced IDO and kynurenine pathway activation.

The role of the kynurenine pathway has not been investigated in SARS-CoV-2, although it is highly relevant to the pathophysiology of all of its high-risk fatality conditions, including diabetes, CVD, hypertension, and ageing (66). Activation of the kynurenine pathway is also prevalent in stress, and may underpin the association of racism/discrimination stress, with SARS-CoV-2 severity and fatality. Stress, including via increased gut permeability/dysbiosis, raises levels of pro-inflammatory cytokine production and therefore IDO and the kynurenine pathway. Stress may also induce the kynurenine pathway via the activation of tryptophan 2,3-dioxygenase (TDO) (67). As such, one of the commonalities of the high-risk medical and social conditions with SARS-CoV-2 pathophysiology will be the propensity to induce IDO and TDO, thereby increasing kynurenine and AhR activation, including in platelets, where it will contribute to platelet aggregation, as well as thrombin and fibrin production. It is such processes that are thought to increase the risk of CVDs in depressed patients. Clearly, the role of the kynurenine pathways requires investigation in SARS-CoV-2 infection.

A corollary of increased IDO, TDO and kynurenine pathway activation is the decreased activation of the melatonergic pathway that ensues. Lower levels of melatonergic pathway activity not only have relevance for regulation of the circadian rhythm by pineal melatonin, but more importantly, will suppress the melatonergic pathway in the mitochondria of possibly all body cells, with consequences for mitochondrial function. Suppression of the mitochondrial melatonergic pathway significantly impacts on the immune response, as it is the induction of the melatonergic pathways in immune cells that leads to the autocrine effects of melatonin that switch immune cells from an M1-like pro-inflammatory state to a more quiescent, M2-like phenotype (68). As such, IDO, TDO and kynurenine pathway activity may be relevant effectors of the maintained 'cytokine storm' that is evident in SARS-CoV-2 infection and many other viral infections, contributing to the pro-inflammatory

pathophysiology as well as platelet activation, and SARS-CoV-2 severity and fatality. The incorporation of the kynurenine pathway allows for a fuller understanding, as well as targeted treatment, of SARS-CoV-2 patients. Higher levels of neopterin, kynurenine, kynurenine-to-tryptophan ratio, and lower levels of tryptophan are evident in influenza patients, where they correlate with severity and poorer outcomes (60, 69). These would be relatively simple measures to be taken in SARS-CoV-2 patients, which would help to shape more targeted treatment response.

It should also be noted that raised IDO levels are evident in epithelial cells and may modulate SARS-CoV-2 entry and consequences, as has been shown for increased IDO in nasal epithelial cells to influenza infection (70). The relevance of the cytokine-IDO/TDO-kynurenine-AhR pathway in pulmonary epithelial cells in response to SARS-CoV-2 will be important to determine. Classically, an increase in IDO is associated with dendritic cell suppression of pro-inflammatory processes. However, as shown for kynurenine activation of the AhR in platelets, this pathway can be associated with significant inflammatory consequences (Figure 1).

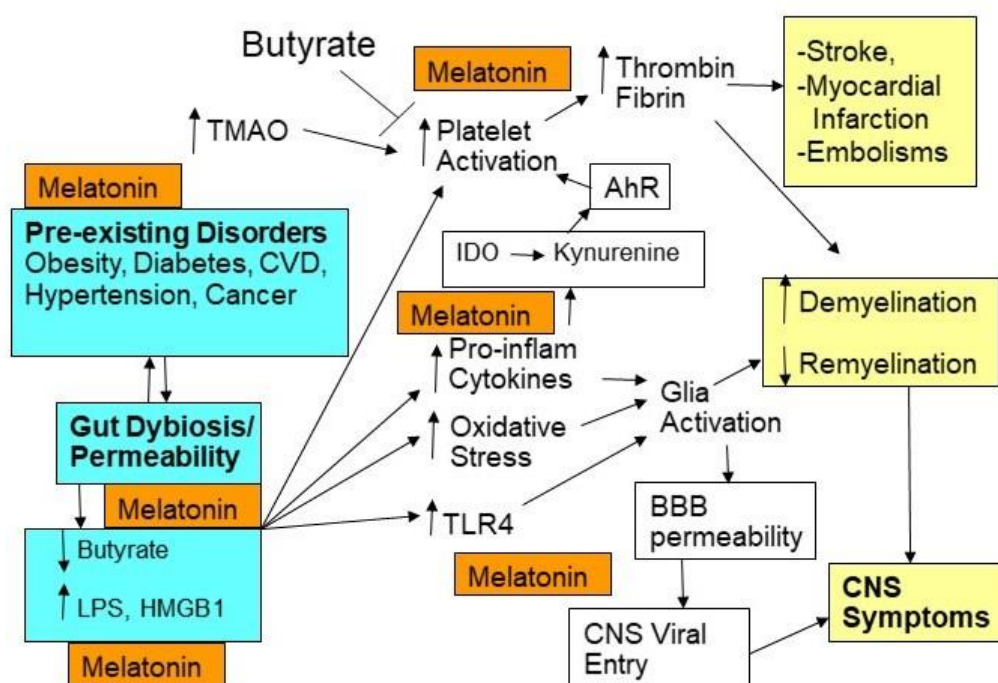


Fig. 1. Potential associations of melatonin with the risk factors presented in the COVID-19.

Pre-existent medical conditions are in two-way interactions with gut dysbiosis/permeability, via decreased butyrate and increased circulating LPS and exosomal HMGB1 to modulate platelet activity, thrombin and fibrin(ogen), thereby impacting on coagulation/embolism associated fatalities from SARS-CoV-2 infection. Pre-existent medical conditions prime wider body systems to effect the same changes induced by SARS-CoV-2. An important aspect of this is the increased pro-inflammatory cytokines, which induce IDO and kynurenine, leading to AhR priming of platelets for aggregation, as well as thrombin and fibrin(ogen). This also increases demyelination and BBB permeability, thereby leading to CNS symptoms and easing viral entry in the brain. Butyrate suppresses platelet activation, whilst the increased TMAO in obesity/diabetes will potentiate platelet activation. The places where melatonin suppresses high risk factors and virus-driven pathophysiology are shown. Melatonin's proposed inhibition of viral entry and the downstream effects via the $\alpha 7nAChR$ are not shown for clarity.

8. AUTONOMIC NERVOUS SYSTEM AND COVID-19

The pro-inflammatory cytokines of the 'cytokine storm' drive viral infection severity and fatality, being associated with heightened sympathetic nervous system activity (71). The role of the autonomic nervous system has been long appreciated in the emergence of pulmonary pneumonia following influenza virus infection (72). There is a growing appreciation of wider impacts of alterations in the renin-angiotensin system and autonomic nervous system in SARS-CoV-2 (73).

Activation of the parasympathetic nervous system, via vagal nerve acetylcholine release, activates the $\alpha 7$ nAChR, where it affords protection against a number of viral infections, including via regulation of immune cell responses (74). As highlighted above (28), vagal ACh release and $\alpha 7$ nAChR activation also underpins melatonin's maintenance of the intestinal barrier under challenge, highlighting the role for variations in autonomic nervous system balance in the regulation of wider aspects of SARS-CoV-2 pathophysiology. Such data may have wider implications. By suppressing pineal melatonin synthesis, SARS-CoV-2 alters the balance of wider body systems, including melatonin's effects via the vagal nerve and the circadian-regulated levels of the $\alpha 7$ nAChR, thereby dysregulating interactions of the parasympathetic nervous system and immune system.

Overall, although not extensively investigated to date, the shift in the sympathetic/parasympathetic nervous systems balance is another aspect of SARS-CoV-2 pathophysiology that will impact on levels of severity and fatality. The autonomic nervous system is also altered in many of the high-risk conditions associated with SARS-CoV-2 symptom severity and fatality (75).

9. VITAMIN D AND COVID-19

As indicated above, a decrease in vitamin D may contribute to SARS-CoV-2 fatalities, perhaps especially in people with higher melanin skin pigmentation (76). As vitamin D regulates immune responses and the lung response to LPS infection (13), there is clearly a role for vitamin D in the modulation of SARS-CoV-2 susceptibility and severity. A number of factors link to this. The emergence of SARS-CoV-2 during the north hemisphere winter is at a time when vitamin D levels are lowest in this population, especially in people with higher melanin skin pigmentation. The extent of COVID-19 pandemic in the southern hemisphere is far lower, as are fatalities. A decrease in vitamin D levels is common over age, thereby plausibly contributing to the high levels of fatalities in the elderly, both in home and care settings (14). Decreased vitamin D also contributes to an increase in gut permeability/dysbiosis, thereby also acting indirectly via the gut to regulate immune and epigenetic responses. Such data would strongly suggest a role for decreased vitamin D in the regulation of SARS-CoV-2 susceptibility and severity, and would suggest utility of vitamin D supplementation, especially under conditions of Lockdown, where exposure to sunlight is often limited.

10. INTEGRATIVE MODEL

10.1. Viral entry and prodromal factors.

The loss of pineal melatonin over ageing, as well as in high-risk conditions for SARS-CoV-2 infection fatality, such as type II diabetes, obesity, CVD, and hypertension, suggests a role for decreased melatonin in both the susceptibility to, and severity of, SARS-CoV-2 infection. Stress, in its many manifestations, including discrimination stress and that arising from

concurrent medical conditions, are often associated with sleep dysregulation and decreased pineal melatonin production. Stress and the high-risk SARS-CoV-2 fatality medical conditions are strongly associated with an increase in gut permeability/dysbiosis, leading to elevations in circulating LPS and HMGB1, which activate TLR4 to decrease pineal melatonin synthesis. The decrease in gut microbiome-derived butyrate leads to an increase in pro-inflammatory immune responses and decreased anti-viral cytotoxicity of natural killer cells, contributing to pineal melatonin suppression and an increase in viral survival and proliferation. As melatonin and butyrate increase the cytotoxicity of natural killer cells, suppressed pineal melatonin release and gut dysbiosis will also attenuate the anti-viral efficacy of natural killer cells. The heightened levels of circulating and central A β in the elderly, including those presymptomatic of any cognitive loss/dementia will also activate TLR4 in microglia and pinealocytes to decrease pineal melatonin production, whilst the loss of pineal melatonin increases A β production. An increase in A β 1-42 will bind the α 7nAChR, attenuating its protective effects, including in the immune system, mitochondria and pulmonary epithelial cells. The loss of the anti-inflammatory, antioxidant, immune-regulatory and mitochondria-optimizing effects of melatonin will contribute to many facets of health that are relevant to responses to viral infection.

The α 7nAChR is one of the important mediators of pineal melatonin effects (22). Suppressed pineal melatonin lowers α 7nAChR levels over the circadian rhythm and its many protective and regulatory effects, thereby heightening the impact of SARS-CoV-2 infection, including its entry into pulmonary epithelial cells. The α 7nAChR is highly expressed in the lung within lipid rafts, where it affords protection to LPS (24). Smokers have a two-fold increase in lung epithelial cell nitric oxide synthase (NOS) (77) and even higher increases in α 7nAChRs, which is proposed to underpin the decreased susceptibility of initial SARS-CoV-2 infection in cigarette smokers. The activation of the α 7nAChR increases NO production, which is proposed to limit the entry of SARS-CoV-2, as previously shown in the original SARS-CoV (78). These authors show NO to inhibit the replication of the original SARS-CoV via two mechanisms: i) reducing the palmitoylation of nascently expressed spike (S) protein, thereby impacting on the fusion between the S protein and ACE2 receptor; and ii) NO reducing viral RNA production in the early steps of viral replication likely via effects on cysteine proteases. Regulation of proteases seems crucial to SARS-CoV-2's ability to appropriately bind the ACE2 receptor and access pulmonary epithelial cells.

As α 7nAChR activation raises NOS levels in many different cell types (79), the protective role of cigarette smoking may be mediated via α 7nAChR contributing to the increased NO that is evident in smokers' lungs (77). The sequence identity of the receptor binding domains of SARS-CoV-2 S protein to ACE2 receptor has 73% homology with that of the original SARS-CoV (80), and it requires investigation as to whether there is a role for NO in the modulation of SARS-CoV-2 entry via the ACE2 receptor. The increase in ACE2 receptors in cigarette smokers would seem to be compensated for by a significant increase in α 7nAChRs, leading to a decrease in viral entry, possibly involving an increase in NO and NO-linked protease regulation, and therefore the cleaving of the SARS-CoV-2 S protein. It is unknown as to the number of proteases that may be involved in S protein cleavage in SARS-CoV-2, with some suggestion of a role for plasmin (81), as well as the main protease for SARS-CoV-2, Mpro. Of note, NO is a significant negative modulator of plasmin activity (82). The effects of dupa7/CHRFAM7A in occluding the protection afforded by the α 7nAChR in pulmonary epithelial cells requires investigation, especially as to the factors that act to regulate dupa7 in pulmonary epithelial cells, as well as other cell types.

Melatonin also affords considerable protection in pulmonary epithelial cells across a host of diverse medical conditions, including COPD and lung cancers, with effects that include the optimization of mitochondrial function (83). As a consequence, it is highly likely that

melatonin will modulate the wider composition of the lipid rafts that contain the ACE2 and $\alpha 7nAChR$ receptors. Clearly, this will be important to ascertain, including as to whether the composition of the lipid rafts modulates ACE2 receptor binding domain structure, as can arise in ligand independent transactivation following neighbouring receptor or channel activation/internalization. It is also of note that melatonin sits on the surface of lipid rafts where it acts to decrease the 'vibrational spaces' evident between lipid components (84). There is a growing appreciation that melatonin can bind a wide array of receptors and channels, occasionally leading to receptor activation, but always impacting on the surface structures that are available for interaction with ligands and other factors (85). As to whether this is relevant in the regulation of the ACE2 receptor and its ability to bind SARS-CoV-2 S protein requires investigation. As well as regulating gut permeability/dysbiosis (12), vitamin D has significant impacts on the immune system and on the anti-viral responses of pulmonary epithelial cells to respiratory virus (rhinovirus) infection (13). A vitamin D receptor, protein disulfide-isomerase associated 3 (PDIA3), is stress regulated and expressed within lipid rafts in pulmonary epithelial cells (86), suggesting an impact on lipid raft complex formation and associated signaling. It is unknown as to whether vitamin D acts to regulate the $\alpha 7nAChR$. However, vitamin D does upregulate ACE2 receptors in a concentration dependent manner, including in response to pulmonary LPS infection (87). It should be noted that ACE2 receptor overexpression attenuates LPS-induced acute respiratory distress syndrome via extracellular signal-regulated kinase/nuclear factor- κB (NF- κB) inhibition (88). Such data highlights an important role for ACE2, including from its induction by antihypertensive medication, indicating that ACE2 levels are important to maintain, whilst blocking its interaction with SARS-CoV-2 S-protein is crucial in preventing virus entry.

Vitamin D also maintains the integrity of the pulmonary epithelial barrier via the maintenance of tight junctions under LPS challenge (89), as well as inhibiting the induction of the small GTPase, RhoA (90). It will be important to determine as to whether vitamin D has any impact on the protease cleavage of the SARS-CoV-2 S-protein. Vitamin D, and its metabolism by CYP24A1, may also have a role in regulating the IDO and the kynurenine pathway, as suggested by a study looking at HIV-1 infection in Ugandans (91). This is also suggested in a study looking at the effects of vitamin D supplementation in general anxiety disorder patients, where vitamin D increased serotonin/melatonin and decreased neopterin levels (92). Such data would indicate that stress, including discrimination stress, may be interacting with decreased vitamin D to modulate processes relevant to SARS-CoV-2 severity and fatality as well as susceptibility. The interactions of vitamin D with kynurenine-AhR associated inflammation and coagulation will be important to determine. As vitamin D has differential interactions with sex hormones, it will be also important to investigate the role of vitamin D in sex differences regarding SARS-CoV-2 infection fatalities (93).

It is also important to note that vitamin D modulates pathways involved in melatonin production, with both acting to optimize mitochondrial function, and thereby impacting on all body systems (94). Interestingly, melatonin binds to the ligand-binding domain of the vitamin D receptor, with melatonin being taken up into cells by the vitamin D receptor, which is blocked by the presence of high vitamin D levels (95). Such data highlight the interactions of melatonin and vitamin D, suggesting that the vitamin D receptor may be differentially used by both over the circadian rhythm. The relevance of this to the regulation of the $\alpha 7nAChR$ and SARS-CoV-2 remains to be determined.

There is still a pervasive bias in Western medicine to conceptualize conditions on the basis of end-point changes in a particular organ or tissue. As indicated above, SARS-CoV-2 infection involves significant changes and interactions with different organs, tissues and body systems. This is also relevant to viral entry, high-risk symptoms and prophylactic treatment. For example, one of the beneficial effects of vitamin D in pulmonary epithelial cells is to

increase levels of the antimicrobial, cathelicidin, which is attenuated by pro-inflammatory cytokines (96). However, the gut-derived short-chain fatty acid, butyrate (and its dietary form, sodium butyrate) can also increase cathelicidin in pulmonary epithelial cells, via histone deacetylation inhibition (97). Sodium butyrate also inhibits the influenza virus induction of pro-inflammatory cytokines in pulmonary epithelial cells (98). Such data would suggest that this is another route whereby the premorbid gut dysbiosis/permeability associated with stress and high-risk COVID-19 susceptibility conditions, may act to modulate SARS-CoV-2 entry and symptom severity. Consequently, prophylactic treatment for SARS-CoV-2 entry and severity may involve targets outwith the lung and based on knowledge of interacting systemic systems.

10.2. Prophylactic treatment.

Prophylactic treatment may be achieved by optimizing night-time melatonin production (e.g. with between 2-10mg melatonin, about 20 minutes before bed-time). The maintenance of gut barrier and a shift to increased butyrate production can be achieved by taking the nutraceutical, sodium butyrate, as recommended (usually with morning and evening meals). This is likely to have beneficial effects across a host of medical conditions, including those associated with high-risk fatality to SARS-CoV-2 infection. Taking vitamin D supplements will afford benefits in immune response and gut dysbiosis/permeability as well as positively regulating pulmonary epithelial cell protective responses to challenge. Targeting the $\alpha 7nAChR$ can be achieved by a number of means, including the utilization of $\alpha 7nAChR$ pharmaceutical agonists, as well as nicotine products, such as gum and patches. As to whether vaping products would better achieve pulmonary $\alpha 7nAChR$ activation seems likely, although requires investigation. As to how nicotine patches, which provide nicotine over 24 hours would better interact with night-time melatonin's induction of the $\alpha 7nAChR$ seems not unlikely, but requires investigation. For people taking anti-A β antibodies or similar medication, there may be more utility in taking this at mid-day, in order to decrease A β effects on the raised $\alpha 7nAChR$ levels at night induced by melatonin.

It is highly likely that the lifting of Lockdown will lead to local SARS-CoV-2 epidemics arising. As such, the maintenance of the above prophylactic treatments may be important to maintain over a number of years. Given the general beneficial effects that will arise, including potentially a decrease in the risk of dementia, diabetes, depression and CVDs, this would not seem burdensome to maintain and should be encouraged by appropriate pricing of products, as well as making melatonin more readily available in countries where it is only available on prescription.

10.3. SARS-CoV-2 symptomatology.

As indicated above, a wide array of body systems show alterations following SARS-CoV-2 infection, with relevance to specific symptoms, severity and fatality. Pre-infection conditions, including diabetes, obesity, hypertension, CVDs, and stress, as well as vitamin D, melatonin, and gut dysbiosis/permeability, will contribute to pre-existent alterations in immune responses, platelet activity, autonomic activity and wider body systems interactions that underpin the variable pathophysiology of SARS-CoV-2 infection across individuals.

Clearly, the initial pro-inflammatory 'cytokine storm' is a major determinant of symptom severity, with effects that seem likely to include an increase in gut dysbiosis/permeability, decreased butyrate and increased circulating LPS and exosomal HMGB1, with consequences for immune regulation, platelet activity, time-to-thrombin and thrombo-embolisms. The rise in pro-inflammatory cytokines will also decrease pineal melatonin release, as will LPS,

HMGB1 and high A β 1-42 levels, with suppressed melatonin having a number of consequences, including: the loss of immune cell dampening and night-time resetting of the immune system; loss of circadian regulation of α 7nAChR levels and effects; an attenuation of Bmal1-induced circadian regulation of mitochondrial OXPHOS, and TCA cycle ATP, coupled to a decrease in acetyl-CoA as a necessary substrate for mitochondrial AANAT; loss of melatonin's antioxidant effects and its induction of endogenous antioxidants; an increase in platelet ROS and activation, leading to increased thrombin and fibrin(ogen) effects that contribute to fatalities, BBB permeability and brain demyelination; the loss of melatonin's regulation of the sympathetic/parasympathetic balance of the autonomic nervous system; loss of melatonin's suppression of pro-inflammatory cytokines, including during the 'cytokine storm'; loss of melatonin's covering of lipid rafts and its interactions with a wide array of diverse receptors, including the vitamin D receptor; and the loss of melatonin's maintenance of the gut barrier, via the vagal nerve and α 7nAChR activation (28).

The increase in pro-inflammatory cytokines will induce IDO and TDO, leading to raised levels of kynurenine and kynurenic acid, which activate the AhR, thereby contributing to SARS-CoV-2 symptoms, including via AhR priming of platelets for heightened activation and coagulation. Activation of the cytokine-IDO-kynurenine-AhR pathway may also be relevant to SARS-CoV-2 entry and effects in different cells, as has been shown for the influenza virus. This is also applicable to vitamin D effects in the regulation of the rhinovirus (13).

The poor understanding of viral interactions with wider body systems, and the relevance of this to SARS-CoV-2 is highlighted throughout. Clearly, future research has to clarify the regulation of the dup α 7/CHRFAM7A and the role of the dup α 7/CHRFAM7A in the regulation of melatonin's induction of the α 7nAChR circadian rhythm. The above has highlighted the role of gut dysbiosis/permeability as primarily mediated via its regulation of the immune system. However, there is a growing appreciation of the relevance of interactions of the gut microbiome with other organ/tissue microbiomes, including the pulmonary microbiome and the relevance that this has for the functioning of the lung and gut (99). Hypertension and associated increased risk of pulmonary embolisms are major contributors to COVID-19 fatalities. Both hypertension and pulmonary embolisms are significantly regulated by ACE2, given its intimate role in the regulation of the renin-angiotensin system. By converting angiotensin II (a vasoconstrictor) to angiotensin (1-7) (a vasodilator), ACE2 lowers blood pressure, allowing ACE2 to have long-appreciated role in the regulation of hypertension. Recent work shows elevations in angiotensin II and ACE, coupled to decreased ACE2 and angiotensin (1-7) to be important in the pathophysiology of pulmonary embolism (100). Such data would suggest a significant role for the regulation of the small GTPases in the treatment of such fatality-associated pathophysiological processes, as indicated in the treatment section below.

10.4. Treating SARS-CoV-2 symptomatology.

It is becoming increasingly clear that the WHO four preferred treatment options (Remdesivir, Lopinavir-ritonavir, (hydroxy)hydrochloroquine and β -interferon) are distractions whilst everyone awaits vaccine development. The process has entailed using products with some, if limited, previous antiviral effects and hope to be lucky. There has been no attempt to integrate current knowledge on body systems that interact with and regulate viral infections. A variety of other factors, from traditional Chinese and Indian medicine are being tried in mostly small scale studies. To date, nothing has shown significant anti-viral benefits against SARS-CoV-2.

In the UK, the national institute for clinical excellence (NICE) has published treatment

guidelines that are based on symptom management e.g. antipyretics for fever and similar focused treatments for cough, breathlessness, anxiety, and pneumonia where present. This is a focus on isolated symptom management.

The above overview of SARS-CoV-2 interactions with various human physiologies would suggest a number of treatment options:

1. Melatonin may have utility regarding broad aspects of symptom management, including the 'cytokine storm' associated fever. Typically, melatonin 2mg/night is recommended for 'jet lag'. However, doses as high as 1000mg have been investigated over 28 days in humans volunteers, with no adverse side-effects, other than drowsiness and vivid dreams (101). Extrapolating from animal studies, it would be recommended that melatonin at 5-10mg/kg would have utility in fever regulation, thereby limiting the downstream consequences of fever in SARS-CoV-2 patients. This may be achieved via an initial dose of 2-3mg/kg, with subsequent doses aiming to maintain melatonin levels. Melatonin is also metabolized by IDO (102), suggesting that IDO induction will also lower melatonin availability, and attenuate melatonin's inhibition of platelet activation, thereby potentiating AhR priming of platelets and associated coagulation. As well as inhibiting platelet activation (103), melatonin also increases platelet levels in thrombocytopenia (104), thereby correcting the two major changes associated with platelets during SARS-CoV-2 infection. As well as monitoring cytokine levels, it will be interesting to monitor wider changes, including platelet activation over the course of melatonin administration.

2. The use $\alpha 7nAChR$ agonists, pharmaceutical or nicotine based, will modulate the immune response, and utilize $\alpha 7nAChR$ induction by high dose melatonin. As detailed above, this will have broad effects, including centrally, as well as in the immune system, gut, platelets and lung.

3. A broad range of vitamins would normally be administered. However, it would be important to have optimal vitamin D, as indicated above.

4. Patients receiving medication targeted to $A\beta$ may benefit from having this medication maintained, given the negative impacts of $A\beta$ on the $\alpha 7nAChR$.

5. It is unclear as to whether sodium butyrate would afford benefit during symptom management. Clearly, pro-inflammatory cytokine induced gut permeability/dysbiosis may contribute significantly to a variety of SARS-CoV-2 symptoms, with data indicating that sodium butyrate can increase the virus-killing, cytotoxicity of natural killer cells. However, sodium butyrate is a powerful epigenetic regulator that will have diverse effects on physiological function.

6. Recent work shows the utility of inhibitors of the small GTPase, RhoA, and its downstream effector, Rho kinase (ROCK), in the management of infection induced acute lung injury and the associated oedema that is evident in the lungs of SARS-CoV-2 infected patients (105-6). Melatonin inhibits RhoA/ROCK induction (107). The effects of RhoA are often opposite to that of another small GTPase, Rac1, with both being induced by sphingosine-1-phosphate (S1P) receptors, S1P2/3r and S1P1r, respectively. Recent data shows S1P to significantly modulate viral infections (108). As S1P r subtypes have significant differential impacts on lipid raft reorganization (109), it will be important to determine the interactions of ACE2 receptors and $\alpha 7nAChRs$ with S1Pr subtypes and $\text{dup}\alpha 7/CHRFAM7A$ regulation in pulmonary epithelial cells and immune cells in SARS-CoV-2 pathophysiology.

11. CONCLUSION

The complexity of various systemic systems and their interactions, including circadian, intestinal, circulatory and immune systems will contribute to the considerable variations evident in SARS-CoV-2 symptomatology. There would seem to be a powerful role for pineal

and local melatonin in the regulation of SARS-CoV-2, including via melatonin's circadian regulation of the $\alpha 7nAChR$. The $\alpha 7nAChR$ in pulmonary epithelial cells is important to the susceptibility as well as symptom severity/fatality in SARS-CoV-2 patients. Both melatonin and the $\alpha 7nAChR$ have treatment implications, both as prophylactics and in symptom management. Stress, vitamin D, platelets, thrombin, BBB permeability, gut dysbiosis/permeability are features of SARS-CoV-2 and it is alterations in such factors that increases SARS-CoV-2 induced fatality in high-risk conditions. There are a number of readily achievable treatment implications arising from embracing the complexity of physiological processes underpinning human interactions with SARS-CoV-2.

ACKNOWLEDGEMENTS

None declared

AUTHORSHIP

GA conceived and wrote this manuscript; RJR over-viewed manuscript for submission.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Please cite this paper as:

Anderson, G. and Reiter, R.J. 2020. COVID-19 pathophysiology: interactions of gut microbiome, melatonin, vitamin D, stress, kynurenine and the alpha 7 nicotinic receptor: Treatment implications. *Melatonin Research*. 3, 3 (Jun. 2020), 322-345. DOI:<https://doi.org/https://doi.org/10.32794/mr11250066>.