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Review article

Lungs as target of COVID-19 infection: Protective common molecular mechanisms of vitamin D and melatonin as a new potential synergistic treatment

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ABSTRACT

COVID-19 pandemic has a high mortality rate and is affecting practically the entire world population. The leading cause of death is severe acute respiratory syndrome as a consequence of exacerbated inflammatory response accompanied by uncontrolled oxidative stress as well as the inflammatory reaction at the lung level. Until now, there is not a specific and definitive treatment for this pathology that worries the world population, especially the older adults who constitute the main risk group. In this context, it results in a particular interest in the evaluation of the efficacy of existing pharmacological agents that may be used for overcoming or attenuating the severity of this pulmonary complication that has ended the lives of many people worldwide. Vitamin D and melatonin could be good options for achieving this aim, taking into account that they have many shared underlying mechanisms that are able to modulate and control the immune adequately and oxidative response against COVID-19 infection, possibly even through a synergistic interaction. The renin-angiotensin system exaltation with consequent inflammatory response has a leading role in the physiopathology of COVID-19 infection; and it may be down-regulated by vitamin D and melatonin in many organs. Therefore, it is also essential to analyze this potential therapeutic association and their relation with RAS as part of this new approach.

1. Background

Facing a lack of a specific treatment against the lethal COVID-19 pandemic, its rapid advance, and taking into account that an adequate immune response is crucial to overcome this viral infection, the need arises to explore pharmacological existing and known agents that reinforce or enhance the immune system activity. The physiopathology of COVID-19 infection and the main cause of death in patients infected with this virus consist of an exacerbated inflammation (with infiltration of immune cells, necrosis, and hyperplasia of affected tissue), especially in the lung level; this results in impaired pulmonary oxygen exchange, causing severe pneumonia [1–3]. Some systemic alterations also are observed, mainly in patients over 60 years, which include disturbance of normal plasma levels of lymphocytes, thrombocytes, C-reactive protein, and lactate dehydrogenase enzyme [4]. Of particular interest, is that older adults are at a higher risk of death [5].

Angiotensin II plasma levels were found significantly elevated in infected patients and were directly proportional to the viral load and lung damage observed [6]. Hence, there are close connections between COVID-19 and renin-angiotensin system (RAS). It has been shown that COVID-19 binds to angiotensin-converting enzyme 2 receptors (ACE2r) to invade human lung epithelial cells and to initiate the infection. At the same time, ACE2 produces anti-inflammatory, antioxidant, anti-fibrotic and anti-hyperplasia effects, since cause the degradation of angiotensin II (Ang II) at lung level by ACE2/Ang1-7/Mas receptor signaling pathway, the counter-regulatory RAS axis with actions opposite to classical RAS axis (ACE/Ang II/AT_1 receptor pathway). The increase in the degradation of Ang II prevents its toxic over-accumulation, which

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would provoke the acute respiratory distress syndrome often present in COVID-19 infection. Therefore, ACE2 has an antagonistic dual action in this viral infection. Also, the expression of ACE2 is lower in males than in females and also lower in older adults than in young people, which could influence the susceptibility of elderly males to death by COVID-19 infection [7–13]. To highlight, this group of patients has a worse prognosis when they are associated, in addition to the elderly condition, with comorbidities such as cardiovascular diseases, diabetes, hypertension, and obesity, all of them with stimulated RAS.

It has also been reported that RAS is involved in the regulation of proliferation, inflammation, and fibrosis of lung tissues in several pulmonary pathologies, independently of COVID-19 infection, such as acute lung injury, asthma, pulmonary arterial hypertension, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis, among others [14]. In this context, it is known that vitamin D and its metabolites enhance immunity to a great variety of respiratory pathogens *in vitro*. Moreover, many clinical trials have been demonstrated a strong association between vitamin D deficiency and increased risk of developing respiratory infections and that its supplementation reduces the events related to respiratory tract infections [15–18]. Other clinical trial results showed that vitamin D supplementation markedly reduces the rate of moderate and severe chronic obstructive pulmonary (COP) disease exacerbations in patients with basal 25-hydroxyvitamin D levels lower than 25 nmol/L but not in patients with higher levels [19]. It was observed that vitamin D decreases the risk of respiratory tract infections by three main mechanisms: the maintenance of tight junctions to prevent the infiltration of immune cells in lungs and other respiratory tissues, the kill of some viruses through the stimulation of antiviral mechanisms, and the reduction in synthesis of pro-inflammatory cytokines through the modulation of the immune system, avoiding the development of pneumonia [20]. In this sense, it is known that vitamin D metabolites modulate the secretion and expression of several chemokines and pro-inflammatory cytokines such as type 1 interferon (IFN-1), CXCL8, CXCL10, TNF-alfa and IL-6 in different viral infections [21].

The antiviral effects of vitamin D through the modulation of the immune system have also been recently suggested. In fact, vitamin D deficiency may be associated with a higher risk of getting influenza, other respiratory viral infections, dengue, hepatitis, herpesvirus, and even human immunodeficiency virus (HIV) infection. In cell culture studies, vitamin D has antiviral efficacy, especially facing enveloped viruses; therefore, it would be effective against COVID-19. Although the antiviral mechanisms of vitamin D have not been completely established, they may be related to the ability of vitamin D to up-regulate the anti-microbial peptides, human beta-defensin 2 and LL-37 cathelicidin [22,23]. Also, the evidence shows that melatonin influences, both directly and indirectly, the immune system state [24]. Simultaneously, melatonin has also been suggested as an antiviral hormone. The effects are exerted through different mechanisms related to melatonin's antioxidation, immunomodulation, and anti-inflammation actions [25]. A recent study proposed the use of melatonin for the treatment of COVID-19 using repurposed drugs, this represents an attractive drug strategy using currently available drugs; this would reduce the time and costs in comparison with *de novo* drug development. The antioxidant properties of melatonin would make it an appropriate candidate drug to relieve the clinical symptoms of patients infected with COVID-19, although melatonin cannot interrupt the replication or transcription of this virus.

The administration of melatonin may prolong the survival time of infected patients, which indicates the possibility that the immune system of these patients recovers due to elimination of the virus, since melatonin indirectly targets several human coronavirus cellular vital points, such as ACE2, BCL2L1, JUN, and inhibitor of nuclear factor Kappa B kinase subunit beta (IKBKB) [26]. Melatonin has also demonstrated its antioxidant and anti-inflammatory effects in mice infected with a respiratory syncytial virus, where it reduced the levels of TNF-alfa significantly, nitric oxide (NO), malondialdehyde (MDA) and hydroxyl radical (·OH), whereas it increased glutathione peroxidase (GSH) and superoxide dismutase (SOD) activity. Therefore, melatonin reversed all altered inflammatory and oxidative parameters of this respiratory viral infection, which suggests it also may be useful in the treatment of COVID-19 infection [27]. Additionally, another study that suggested the use of melatonin in the management of COVID-19 infection has also highlighted the high safety profile of this hormone, which encourages, even more so, its clinical application [28].

It is also known that with advancing age not only causes a decline in immunity but also in melatonin and endogenous vitamin D production. Therefore, this could also explain the greater susceptibility of older adults to death by COVID-19 infection [29]. It could also be hypothesized that there is a lower infection rate in older women because more of them are undergoing treatments for bone diseases that incorporate vitamin D supplements, although there is no scientific evidence that supports this.

All this evidence supports the beneficial effects of vitamin D and melatonin as being helpful against lung pathologies in pulmonary infection by COVID-19, where there is also exacerbated inflammation, oxidation, and other similar pathophysiologic mechanisms. This information suggests using analyzing both these natural compounds and well as their possible synergism to overcome this pandemic that affects many people around the world while searching for definitive treatment or vaccine continues. Additionally, it is essential to assess the potential relationship between both compounds and the renin-angiotensin system as a mechanism of cell entry of COVID-19 to establish potential treatments that avoid the first step in this viral infection.

2. Vitamin D and its pulmonary anti-inflammatory and antioxidant actions

It has been suggested that the deficiency of vitamin D is usually associated with the hyper responsiveness of airways, impaired pulmonary functions, worse control of asthma, and perhaps resistance to steroids. Pulmonary epithelial cells have a high expression of 1 alpha-hydroxylase level, which allows the local synthesis of 1α, 25-dihydroxy vitamin D, the active form of vitamin D, also called calcitriol in the lungs. Calcitriol inhibits the production and secretion of many cytokines from bronchial smooth muscle cells, such as growth factor derived from platelets, RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted), and matrix metalloproteinases, which leads to a reduction in smooth muscle cell proliferation and lung inflammation. Moreover, vitamin D stimulates the synthesis of interleukin 10 by CD4 + CD25 + Foxp3 + T-regulatory cells and dendritic cells. At the same time, vitamin D inhibits the activation of dendritic cells by a downregulating expression of CD80/86 and CD40 as co-stimulatory molecules, and stimulates the expression of many anti-infective molecules [30,31]. 1α, 25-dihydroxy vitamin D supplementation suppresses the recruitment of eosinophil and lymphocyte into the airways, decreases IL-4 production from T cells, and inhibits the migration of T cells, attenuating the inflammatory response [32].

Vitamin D may also be useful as an adjuvant for other therapies such as allergen immunotherapy, whose beneficial effects were improved by simultaneous 1, 25-dihydroxy vitamin D administration to a mouse model of asthma [33]. Vitamin D and dexamethasone treatment administration to cultured CD4þ regulatory T cells from steroid-resistant asthmatic patients increased the synthesis of IL10 to levels similar to those found in steroid-sensitive patients treated only with dexamethasone [34]. In a similar *in vitro* model of steroid resistance, it was observed that vitamin D caused the inhibition of T cell proliferation when dexamethasone alone could not suppress cell proliferation [35]. The high serum levels of IgE and eotaxin found in an asthmatic rat model were also significantly reduced by the treatment with vitamin D [36]. In asthmatic mice, vitamin D treatment also decreased the infiltration of inflammatory cells in airways, the serum levels of IL-6, tumor necrosis factor (TNF) α and (IL) 1β, and the expression of Bcl 2-associated X apoptotic protein, caspase 3 (CASP3), high mobility group

box 1 protein (HMGB1), TLR4, NF κB and phosphorylated NF κB p65. Likewise, vitamin D elevated IL10 serum levels, reducing the inflammatory and apoptotic response in these mice [37]. In human bronchial epithelial cells exposed to pollutant particulate matter, vitamin D suppressed the synthesis of 8-isoprostane (8-iso), IL-6, and granulocyte-macrophage colony-stimulating factor-stimulated by this pollutant agent. Vitamin D caused a rise in the expression of G6PD antioxidant pathway gene and the levels of oxidized glutathione, which suggest that vitamin D, can protect the lungs and airways in asthmatic pathology through its anti-inflammatory and antioxidant effects facing air pollutant exposure [38].

In a murine model of pulmonary inflammation induced by bleomycin, calcitriol reduced early lung inflammation by attenuating immune cell infiltration, repressing pulmonary inflammatory cytokines secretion, blocking nuclear translocation of nuclear factor kappa B (NF-κB) p65, inhibiting phosphorylation of pulmonary p38 MAPK and protein kinase B (Akt), attenuating α-smooth muscle actin (a marker for epithelial-mesenchymal transition in the lungs, which promotes fibrosis), and decreased transforming growth factor-beta 1 (TGF-β1) upregulated and Smad phosphorylation [39]. Calcitriol also caused a reduction of approximately 40% in neutrophil recruitment to lungs in an animal model of acute lung injury, considerably inhibiting this pathological condition. This anti-inflammatory effect of vitamin D may be mediated by the inhibition of IL-8 secretion at the lung level [40]. The administration of vitamin D to neonatal rats with lung injury induced by hyperoxia (as a model of bronchopulmonary dysplasia) caused attenuation of this lesion *via* several protective actions, such as preserving pulmonary structure integrity, decreasing inflammation by downregulating TLR4 activation, reducing the deposition of the extracellular matrix, and inhibiting lung cell apoptosis [41]. Vitamin D has also shown to have immunomodulatory and anti-inflammatory effects in the treatment of airway cystic fibrosis since it reduced the expression of CD279 (PD-1) on CD4+ and CD8+ T cells. Moreover, vitamin D decreased the frequency of CD8+ T and mucosal-associated invariant T cells that co-express the CD38 activation markers and human leucocyte antigen D-related. Therefore, vitamin D treatment would prevent lung damage progression associated with airway cystic fibrosis [42]. The oxidative stress that causes tobacco smoke worsens the progression of chronic obstructive pulmonary disease. In this sense, vitamin D has also been proposed as a natural anti-inflammatory and antioxidant able to improve the prognosis of this lung pathology in smoking patients [43]. In fact, it has been observed that patients with the chronic obstructive pulmonary disease have lower plasma levels of vitamin D than healthy patients, suggesting a possible correlation between a deficient antioxidant defense and development of this pulmonary disease [44]. Of central interest to our revision, some years ago, our group raised the discussion on a world pandemic of vitamin D deficiency as a possible explanation by cellular inflammatory response activity RAS-induced [45]. The original discussion involved a significant number of pathologies -mainly cardiovascular- but with a similar inflammatory basis. At present, with the central focus on the acute pulmonary inflammation caused by COVID-19, the Irish Longitudinal Study on Ageing (TILDA 2020) reinforces the idea that an adequate supplementation of vitamin D, especially in older people, may be beneficial for vulnerable population groups during this COVID-19 pandemic outbreak [46] (Graphical abstract).

3. Melatonin and its effects on inflammation and oxidation at lung level

Therapeutic potential of melatonin at the respiratory system level is mediated, among other mechanisms, by blockade of nuclear factor-κappa beta (NF-κβ), overexpress of c-Fos, and down-regulate of matrix metalloproteinases-3 (MMP-3), which modulates pro-fibrotic and pro-inflammatory cytokines [47]. Moreover, the protective role of melatonin in pulmonary hypertension is due to its antioxidant, anti-fibrotic, and vasodilator properties [48]. It has also been suggested that pretreatment with melatonin in a murine model of asthma reduces the accumulation of collagen in the airways, possibly through the inhibition of matrix metalloproteinase-9, which modulates tissue remodeling. Therefore, melatonin would be expected to exert an anti-inflammatory effect at the level of the airway [49].

Melatonin has also been useful in the protection of pulmonary tissue during acute lung injury in rats due to its ability to eliminate free radicals and block the activation of NF-kappa B [50]. 5-Hydroxy-2′-isobutyl-streptochlorin (HIS), a novel derivative of melatonin with enhanced anti-inflammatory properties, inhibits the penetration of immune cells into the lung and the secretion of pro-inflammatory cytokines such as TNF-α and IL-6 in a murine model of acute lung injury. These anti-inflammatory effects of HIS were mediated by the modulation of the signaling pathway-dependent of interferon-β, and toll-like receptors. Moreover, HIS inhibited the secretion of IL-1β by blocking the activation of NLRP3 inflammasome independent of mitochondrial production of reactive oxygen species [51]. The lung surfactant lipid peroxidation is induced by the production of oxygen radicals from stimulated phagocytes responsible for the development and progression of acute lung injury. In this sense, melatonin alone or combined with other antioxidants markedly reduced lipid peroxidation of this pulmonary surfactant [52]. Intratracheal administration of melatonin provoked a marked reduction of lung lesions and recruitment of neutrophils and macrophages to lungs during acute pulmonary injury. Furthermore, melatonin inhibited the NLRP3 inflammasome activation through the suppression of extracellular histone release [53].

It has been suggested that melatonin inhibits pulmonary fibrosis in animal models through the decrease in the migration of fibroblasts as a consequence of the reduction in the activity of chloride channels mediated by protein kinase C [54]. In an animal model with pulmonary fibrosis induced by bleomycin, melatonin provoked a significant reduction in edema and lesions at the lung level. Likewise, melatonin treatment lowered the expression of cyclooxygenase 2, which produces eicosanoids whose role is crucial in the development of pulmonary fibrosis. Besides, melatonin caused a decrease in interstitial tissue percentage volume and an increase in the alveolar space percentage volume [55]. Melatonin attenuated the pneumonitis and lung fibrosis caused by radiation exposure through a decrease in inflammatory cell infiltration, collagen deposition, edema, and vascular and alveolar thickening in the lungs [56]. Idiopathic pulmonary fibrosis causes the progressive loss of pulmonary function due to tissue cicatrization. Interesting, it has been reported that melatonin and its metabolites can modulate many pro-inflammatory and pro-fibrogenic signaling pathways involved in the pathophysiology of pulmonary fibrosis, such as transforming growth factor β, Wnt/β catenin, interleukin 17A, vascular endothelial growth factor, fibroblast growth factors, platelet-derived growth factor, renin-angiotensin system, endothelin 1, and impaired caveolin 1 function, causing protective effects on lungs [57]. By removing free radicals, melatonin may adequately modulate autophagy pathways and apoptosis, which are key mechanism in the development of idiopathic pulmonary fibrosis [58]. Additionally, the utilization of melatonin in the treatment of animals with induced hepatopulmonary syndrome was effective in the reduction of lung fibrosis levels, vasodilation, and oxidative stress [59].

Melatonin also ameliorates pulmonary lesions associated with inflammation and oxidative stress caused by nitrogen mustard in rodents, since it acts as a powerful antioxidant that eliminates both reactive nitrogen and oxygen species and as a potent anti-inflammatory agent [60–62]. The pretreatment with melatonin combined or not with quercetin in rats with hypoxia-induced by sodium nitrite considerably reduced the plasma levels of IL-6, TNF-α, and CRP, heat shock protein 70 extracellular (Hsp70e), and VEGF. Additionally, melatonin enhanced the histopathological changes in the lungs of these animals, also suggesting powerful protective effects of melatonin at the pulmonary level [63]. Melatonin has a potent pulmonary protective effect in rats

with lung injury induced by phosgene, and this protective mechanism can be associated with the elimination of free radicals and the inhibition of p38 MAPK activation and iNOS expression [64]. Pretreatment with melatonin is effective in reducing lung damage in patients subjected to chemotherapy. Melatonin with cyclophosphamide decrease lipid peroxidation, restoring the glutathione levels, and superoxide dismutase/catalase activities. Furthermore, the mentioned association could reduce the typical histological abnormalities in the lungs of patients with chemotherapy [65].

Melatonin has also inhibited the expression of hypoxia-inducible factor-1α (HIF-1α), proliferating cell nuclear antigen (PCNA), and nuclear factor-κB (NF-κB) in a murine model of hypoxic pulmonary hypertension. This molecule also suppressed the proliferation of pulmonary artery smooth muscle cells, and the concentrations of phosphorylated Akt and kinases 1/2 modulated by extracellular signal provoked by hypoxia in an *in vitro* model. These results also suggest significant antiproliferative and anti-inflammatory effects of melatonin at the pulmonary level [66]. Postnatal administration of melatonin reduced the pathological vascular remodeling and the cardiovascular response to hypoxia in neonatal lambs with pulmonary hypertension. On the other hand, melatonin increased angiogenesis in these animals. These effects enhance the pulmonary vascular function and structure in the neonatal period under conditions of chronic hypoxia [67,68].

Melatonin also markedly inhibited the pulmonary injury induced by hepatic ischemia/reperfusion in rats. Through its anti-inflammatory properties achieved the reduction in apoptosis of lung cells by blocking of JNK, p38 and NF-ƙB activation, and stimulating of Nrf2 activation [69]. In mice with induced lung ischemia-reperfusion, melatonin pretreatment achieved a reduction of the damage in lung parenchymal, and a decrease in the expression of inflammatory markers such as interleukin-1β, TNF-α, and IKK-γ; apoptotic markers such as Bax/Bcl-2 and cleaved CASP3, and TUNEL positive cells were also inhibited. In addition, melatonin pretreatment increased the expression and activity of cellular antioxidant systems superoxide dismutase, glutathione peroxidase, and glutathione reductase [70].

In an animal model of neurogenic pulmonary edema, melatonin attenuated the dysfunction of the alveolar-capillary barrier by inhibiting the disruption of tight junction proteins such as ZO-1 and occludin. Furthermore, it caused a downregulation of myeloperoxidase and interleukin (IL) -1β, as well as provoked an inhibition of the activation of matrix metallopeptidase 9. In addition, the melatonin treatment considerably decreased CASP3 activity and the number of positive lung cells in the TUNEL assay. Therefore, melatonin treatment enhanced the prognosis of neurogenic pulmonary edema due to its anti-inflammatory and anti-apoptotic effects [71]. Melatonin also exerted its anti-inflammatory, antioxidant and anti-apoptotic effects in a murine model of lung aging, significantly reducing the expression of apoptosis markers such as BAX, BAD, AIF, inflammatory markers such as IL-1β, TNF-α, HO-1, NFκB2, and the oxidative damage to RNA measured as the production of 8-hydroxyguanosine [72].

It is well documented that melatonin prevents chronic obstructive pulmonary disease by attenuating NLRP3 inflammasome and IL-1β, responsible for the inflammation of airways during this pathology. Additionally, melatonin caused an increase in silent information regulator 1 (SIRT1) expression, which mediates the mentioned anti-inflammatory effect and other additional protective mechanisms, such as the attenuation of apoptosis and endoplasmic reticulum stress [73,74].

Melatonin caused a decrease in oxidative stress and inflammation of airways and lungs and reduced the chronic cough induced by particulate matter 2.5 μm in a pig model [75]. Additionally, it has been demonstrated that environmental pollution in the form of particulate matter < 2.5 μm stimulated the melatonin synthesis at lung level in order to protect lung tissues from the damage provoked by the inhalation of polluted air [76].

The administration of ramelteon (an agonist of melatonin receptor) in an animal model of lung injury induced by ventilation and notably decreased the pulmonary edema, malondialdehyde concentrations, the levels of proinflammatory cytokines, the activation of NF-κB, the expression of iNOS, and apoptosis in lung cells. Moreover, ramelteon treatment markedly elevated the expression of intracellular protective heat shock protein 70 (Hsp70i) in lung cells and the levels of anti-inflammatory cytokine IL-10 in bronchoalveolar lavage fluid. These results suggest that the protective effects of melatonin on lung disease are mainly mediated by a melatonin receptor [77].

It was documented that exposure to nicotine in pregnant rats caused significant changes in the lungs of their offspring at a structural and biochemical level. In this regard, melatonin treatment reduced the altered parameters by a lowering in the number of alveolar macrophages and mast cells, as well as in the levels of malondialdehyde [78]. Melatonin also significantly reduced the oxidative stress and injury of lungs exposed to radiation through the suppression in the infiltration of neutrophils (CD11b + Ly6G +) and macrophages (CD11b + CD11c-), and the down-regulation of NLRP3 inflammasome in macrophages (accompanied with a reduction in the release of IL-1β and the activity of caspase-1 or CASP1), by the up-regulation of miR-30e levels [79]. Melatonin treatment decreased lung injury in rats exposed to chromium by up-regulating SIRT1, which promotes the deacetylation of the transcriptional co-activator peroxisome proliferator-activated receptor-γ coactivator-1α (Pgc-1α). At the same time, this caused a rise in the expression of crucial antioxidant target genes and the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). These findings indicate that melatonin exerts anti-inflammatory, antioxidant, and anti-apoptosis effects in the attenuation of lung injury provoked by chromium exposure through SIRT1/Pgc-1α/Nrf2 signaling pathway [80]. It has also been determined that melatonin was able to mitigate the lung damage induced by carbon tetrachloride by the elimination of free radicals, the increase in the antioxidant ability of pulmonary tissues, and the induction of an effective anti-inflammatory response [81].

Melatonin also reduced the increased levels of malondialdehyde and myeloperoxidase in the pulmonary tissue of diabetic rats. Moreover, it attenuated the bronchial hyperplasia and reduced the cleaved-caspase 3 expression, which was significantly increased in these animals [82] (Graphical abstract).

Of special interest, a clinical trial about the use of melatonin in the prophylaxis of COVID-19 infection among healthcare workers is carrying out (ClinicalTrials.gov Identifier: #NCT04353128). Moreover, many other human trials have demonstrated the efficacy of melatonin in the reduction of elevated levels of circulating cytokines in multiple inflammatory pathologies, suggesting that melatonin would also useful in the treatment of COVID-19 infection by decreasing plasma levels of proinflammatory cytokines [83–88]. Furthermore, other clinical trials showed that melatonin is effective against chronic obstructive pulmonary disease [89] and multiple newborn diseases associated with increased oxidative stress (at lung levels and in many other organs) due to its important antioxidative properties [90].

4. Melatonin, vitamin D and RAS: a rational protagonist Triade in COVID-19?

It is known that there is a strong interaction between melatonin and RAS [91,92]. In this regard, melatonin treatment caused a reduction in AT1 expression and normalized the Ang II levels in a model of renal disease [93]. It is also known that there is a local pineal RAS that modulates the synthesis of melatonin. In this regard, it has been indicated that Ang II acts on the AT1 receptors located in pineal gland cells to regulate the expression and activity of tryptophan hydroxylase enzyme, which is involved in the synthesis of melatonin. It has also been demonstrated that the antioxidant, anti-inflammatory, and anti-apoptosis effects of melatonin are opposite for Ang II [94]. It has reported that patients with chronic renal disease secrete lower nocturnal melatonin concentrations than healthy patients. This altered secretion may be related to increased nocturnal intrarenal RAS activation and

kidney damage in patients with this renal disease. This fact reinforces the existence of a close antagonistic interaction between RAS and melatonin [95]. In this regard, it is known that reactive oxygen species are crucial in the activation of intrarenal RAS. Also, it has been shown that antioxidant treatment with melatonin ameliorates the intrarenal RAS over-activation and renal injury in a 5/6 nephrectomized rat model of chronic kidney disease [93,96]. Despite the lack of studies that specifically show a relationship between melatonin and RAS at the lung level, the existing evidence of this relationship in other organs such as the kidney suggests the possibility of similar behavior at the respiratory level.

Concerning vitamin D/RAS interaction, the participation of ACE2/Ang(1-7)/MasR signaling pathway in the neuroprotective effects of vitamin D in the brain has recently demonstrated in hypertensive rats [97], and it has found that vitamin D acts as a cofactor in the attenuation of incident atrial fibrillation by RAAS inhibition [98]. Furthermore, the re-establishment of normal vitamin D levels in patients with D hypovitaminosis provokes blockade of peripheral RAS [99]. Exacerbated activation of RAS at the hepatic level causes liver dysfunction and increases the risk of developing diabetes mellitus. In this sense, it has been found that calcitriol modulates the altered up-regulation of the liver RAS in conditions of insulin resistance [100]. Vitamin D is a potent suppressor of renin production. Thus, low plasma levels of vitamin D are associated with an increase in the synthesis of renin, which results in an over-activation of RAS and an increased production of Ang II, and *vice versa* [101,102]. It has been demonstrated that vitamin D deficiency also results in overexpression of angiotensin-converting enzymes (ACE and ACE2) [103].

It has also been observed that vitamin D receptor-null mice develop more serious induced acute lung injury than wild-type mice, with increased levels of pulmonary Ang II and renin. Pretreatment of vitamin D receptor-null mice with losartan reduced the severity of the pulmonary injury, indicating that vitamin D, *via* its receptors, attenuates acute lung injury by blocking RAS [104]. Moreover, if vitamin D deficiency is chronic, the uncontrolled RAS over-activation for extended periods may induce pulmonary fibrosis through the exacerbated and accelerated increase in extracellular matrix deposition in lung tissues [105] (Graphical abstract).

5. Conclusion and prospects

The combined supplementation of vitamin D with melatonin could offer an attractive synergistic alternative for the prevention and treatment of pulmonary infection by COVID-19. These molecules modulate the same signaling pathways that relate to anti-inflammatory, immunomodulatory, antioxidant, anti-fibrotic, as well as anti-apoptotic effects, in many tissues with special focus at the lung level. Both natural compounds are highly safe for clinical use. They have many shared underlying mechanisms that allow them to exert potentiating actions aimed at strengthening the immune system and preparing the body to overcome the severe pathological consequences of COVID-19 infection and, if there is an infection, reduce its high mortality rate.

There exist many therapeutic objectives when evaluating the efficacy of vitamin D and melatonin in the prevention and treatment of COVID-19 infection. However, RAS ends up being the bottleneck, a central signaling pathway, because it constitutes a common meeting point for this triad. RAS stimulation, with the consecutive inflammatory storm, favors the infection, evolution, and outcome of this complex new pathologic entity. As previously suggested, the global deficiency of vitamin D as a result, among others, of cultural changes, could respond mechanistically at least in part to higher inflammatory processes as a consequence of lack of brake that vitamin D exerts typically on the RAS system during healthy conditions [45].

As a perspective, it would be of interest to carry out clinical trials to evaluate the therapeutic utility of the vitamin D/melatonin combination, both in healthy patients and those infected with COVID-19, and to compare the effects of its joint administration *versus* their individual administration. In this regard, it would be specially interesting adequately to define both melatonin and vitamin D supplementation regime used, taking into account both group and individual features of patients that will participate of these studies, since not considering these aspects may lead to obtain heterogeneous and controversial results that could affect their general applicability [106]. However, with the central focus on acute lung inflammation caused by COVID-19, a longitudinal study suggest adequate vitamin D levels in vulnerable groups during this pandemic outbreak by COVID-19 [46]. The last findings showed that the degree of protection against infections increases as vitamin D levels increase, but this relationship has not allowed establishing an adequate cut level yet. Still, an observational study reported that 38 ng/mL are appropriate values to decrease the risk of acute viral respiratory infections [107]. On the other hand, some authors suggest maintaining a serum vitamin D level at least 30 ng/mL or even maintaining it in a range of between 40 and 60 ng/mL to reduce infectious processes. Moreover, recently Dr. Alipio has provided substantial information to physicians and health policymakers. Specifically, it concluded that vitamin D supplementation improves the clinical course of patients infected with COVID-19 based on the increased probability of having a mild result when the serum level of vitamin D increases while a serum decrease in vitamin D is associated with worse clinical evolution [108]. The same recommendation was reinforced by Grant and colleagues who suggested that vitamin D supplementation could reduce the risk of COVID-19 infection [109]. Also, Rhodes and collaborators have proposed vitamin D supplementation, at least for those in the northern hemisphere who are at higher risk of severe illness and death [110]. The same is recommended by the United Kingdom Association of Dietitians [111] and editorials in international scientific rigors.

Finally, although there are some controversies regarding the effectiveness of vitamin D and melatonin in clinical studies; such contradictions are the consequence of experimental designs with many errors. The latest findings -with better designs- begin to show robust evidence of benefits. So, melatonin, on the one hand, and vitamin D, on the other, would be complicit endogenous as physiological responders and/or exogenous drugs in therapeutics treatment, which could alleviate the pathological process even before the infection develops.

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Declaration of competing interest

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