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COVID-19 and Vitamin D: A lesson from the skin

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Abstract

The negative outcomes of COVID-19 diseases respiratory distress (ARDS) and the damage to other organs are secondary to a “cytokine storm” and to the attendant oxidative stress. Active hydroxyl-forms of vitamin D are anti-inflammatory, induce anti-oxidative responses, and stimulate innate immunity against infectious agents. These properties are shared by calcitriol and the CYP11A1-generated non-calcemic hydroxyderivatives. They inhibit the production of pro-inflammatory cytokines, downregulate NF-κB, show inverse agonism on RORγ and counteract oxidative stress through the activation of NRF-2. Therefore, a direct delivery of hydroxyderivatives of vitamin D deserves consideration in the treatment of COVID-19 or ARDS of different etiology. We also recommend treatment of COVID-19 patients with high dose vitamin D since populations most vulnerable to this disease are likely vitamin D deficient and patients are already under supervision in the clinics. We hypothesize that different routes of delivery (oral and parenteral) will have different impact on the final outcome.

Key words

COVID-19, SARS-CoV-2, cytokine storm, oxidative stress, vitamin D, vitamin D-hydroxyderivatives

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Background

The COVID-19 is currently the foremost health issue in the world. SARS-CoV-2 (severe acute respiratory syndrome coronavirus) is an enveloped positive strain RNA virus in the family *Coronaviridae*, which also includes the virus SARS-CoV-1 (which was another outbreak in 2002-2003)[1]. COVID-19 has a fatality rate up to ~5%, which is several times higher than influenza[2,3]. The leading cause of death in the patients is due to acute respiratory distress syndrome (ARDS)[2] induced by proinflammatory responses and oxidative stress (Fig. 1A).

Vitamin D is a fat-soluble prohormone, which after production in the skin or oral delivery affects important physiological functions in the body including regulation of the innate and adaptive immunity[4-6]. Vitamin D can be activated through canonical and non-canonical pathways (Fig. 1A). In the former, it is metabolized to 25-hydroxyvitamin D_3 ($25(OH)D_3$) by CYP2R1 and CYP27A1 in the liver with further metabolism in the kidney to the biologically active 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) by CYP27B1[7-9]. This metabolism also occurs in a variety of organs, including skin and the immune system[7,9].

An alternative pathway of vitamin D activation by CYP11A1 leads to production of more than 10 metabolites some of which are non-calcemic even at high doses[8,10,11]. These hydroxyderivatives, including $20(OH)D_3$ and $20,23(OH)_2D_3$, are produced in humans[12-15]. In addition, $20(OH)D_3$ has been detected in the honey, which defines it as a natural product[16]. CYP11A1 is expressed not only in adrenals, placenta and gonads but also in immune cells and other peripheral organs[17].

Both $1,25(OH)_2D_3$ and non-calcemic CYP11A1 derived metabolites use various, although partially overlapping, mechanisms in enacting their anti-inflammatory and anti-oxidative effects (Figure 1B). $1,25(OH)_2D_3$ mediates many of its anti-inflammatory and anti-microbial effects through the vitamin D receptor (VDR)[6,9]. $1,25(OH)_2D_3$ can also inhibit the mitogen-activated protein kinase (MAPK) and NF-kB signaling[4,9].

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The non-calcemic CYP11A1-derived vitamin D compounds also have their own methods to fight inflammation (Fig. 1B). 20(OH)D_3 and their downstream hydroxyderivatives act on VDR as biased agonists[11,18,19]. They also act as inverse agonists on the retinoic acid-related orphan receptors, RORα and RORγ, transcription factors with critical roles in several immune cells and immune responses[20-23] (Fig. 1B). In addition, CYP11A1-derived derived vitamin D_3 derivatives and classical 1,25$(OH)_2D_3$ can act as agonists on aryl hydrocarbon receptor (AhR)[24]. Although binding pocket of this receptor can accommodate many different molecules, we believe that secosteroidal signal transduction can be linked to detoxification and anti-oxidative action[11] or down-regulation of pro-inflammatory responses[25].

Premises

ARDS and other adverse effects of COVID-19 are induced by cytokine storm

A leading cause of ARDS is "cytokine storm", a hyperactive immune response triggered by the viral infection (Fig. 1A)[2,26]. It is initiated when the pattern recognition receptor of the innate immune cells recognize the pathogen-associated molecular pattern from a pathogen such as bacteria or virus[26,27]. The immune cells then release all types of cytokines (interferons, interleukins 1, 6 and 17, chemokines, colony stimulating factors, and tumor necrosis factor (TNF)) leading to hyperinflammation and organ damage[27-29]. In the lungs, alveolar cells are targeted leading to acute lung injury and subsequently ARDS[27,30]. In severe cases of CoVID-19 other organs and systems are also damaged[2,3]. Thus, it is crucial to find ways to prevent the "cytokine storm" from going out of control. Although different drugs have been suggested to fight the cytokine storm[26,27], they have mixed results and in certain cases can even worsen the disease[27]. Thus, there is a great need for alternative therapies.

Oxidative stress is also involved in the development of ARDS through action of reactive oxygen species (ROS) and nitrogen species (NRS)[31-33]. The production of ROS and RNS can be triggered by pathogens promoting the secretion of cytokines, which stimulate ROS production thereby producing a positive feedback loop (Fig. 1A)[31,33-35]. Nuclear factor erythroid 2p45-related factor 2 (NRF-2) is a

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transcription factor that plays a role in the detection of excessive ROS and RNS and induction of mechanisms counteracting the oxidative damage[36]. NRF-2 loss due to ROS can lead to elevation in proinflammatory cytokine levels and stronger inflammatory responses to stimuli[31,36].

Anti-inflammatory and antioxidative activities of active forms of vitamin D

There is a strong experimental evidence that active forms of vitamin D including the classical $1,25(OH)_2D_3$, and novel CYP11A1-derived hydroxyderivatives[8,11] exert potent anti-inflammatory activities including inhibition of IL-1, IL-6, IL-17, TNFα and INFγ production or other proinflammatory pathways (Supplemental table 1) [11,18,20,37,38]. The mechanism of action includes downregulation of NF-κB involving action on VDR and inverse agonism on RORγ leading to attenuation of Th17 responses (Fig 1B)[11,18,20,37-39]. These compounds also induce antioxidative and reparative responses with mechanism of action involving activation of NRF-2 and p53[11,39-41].

Antiviral effects of active forms of vitamin D

Low vitamin D status in winter permits viral epidemics and vitamin D supplementation could reduce the incidence, severity, and risk of viral diseases[42-45]. In addition, several reports have found a strong association between vitamin D deficiency/insufficiency and enhanced COVID-19 severity and mortality[45-53] with the most recent study defining low plasma $25(OH)D_3$ as an independent risk factor for COVID-19 infection and hospitalization[54]. Therefore, we retrospectively analyzed microarray data of human epithelial cells treated with $20,23(OH)_2D_3$ and $1,25(OH)_2D_3$[24]. We found the downregulation of pathways connected with influenza infection and viral RNA transcription, translation, replication, life cycle and of host interactions with influenza factors with $20,23(OH)_2D_3$ expressing higher anti-viral potency (Table 1).

While $1,25(OH)_2D_3$ has the limitation imposed by the toxicity that includes hypercalcemia[7,9], CYP11A1-derived $20(OH)D_3$, $20(OH)D_2$ and $20,23(OH)_2D_3$ are not toxic and non-calcemic at very high doses (3-60 μg/ kg) at which $1,25(OH)_2D_3$ and $25(OH)D_3$ are calcemic[55-59].

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Hypothesis

The hyperinduction of proinflammatory cytokines production (cytokine storm), further magnified by oxidative stress induced by the viral infection or cytokines themselves, acting reciprocally in self-amplifying circuitry, gradually damage/destroy the affected organs leading to death in the severe cases of COVID-19 infection (Fig. 1A). A solution to the problem fulfilling above premises, are active forms of vitamin D including the classical $1,25(OH)_2D_3$ and $25(OH)D_3$ (precursors to $1,25(OH)_2D_3$)[5,7,9,45,60] and novel CYP11A1-derived hydroxyderivatives including $20(OH)D_3$ and $20,23(OH)_2D_3$[8,11,61]. The former are FDA approved and can immediately be used in the clinic, while the latter are still not approved yet although they fulfill the definition of natural products. They would both terminate "cytokine storm" and oxidative stress with possible anti-viral activity to rescue the patient from the death path (Fig. 1). Their preferable routes of delivery are listed in Fig. 1C to reach immediately the most affected organs. In this context, active hydroxyforms of vitamin D_2 should also be considered[59,62-64].

As relates to the vitamin D precursor it is reasonable to propose that patients being admitted with COVID-19 infection to receive as soon as possible 200,000 IU of vitamin D_2 or vitamin D_3 followed by 4,000-10,000 IU/day, if justifiable[45,65]. Vitamin D3 at 200,000IU orally has been used to attenuate inflammatory responses induced by the sunburn[66]. It must be noted that application of 250,000–500,000 IU of vitamin D was reported be safe in critically ill patients and was associated with decreased hospital length of stay and improved ability of the blood to carry oxygen (reviewed in[67,68])

Relevance and perspective

Different routes of delivery of vitamin D precursor can have a profound effect on the final panel of circulating in the body vitamin D derivatives (Fig. 1C). Vitamin D delivered orally during the passage through the liver is hydroxylated to $25(OH)D$[7], which is not recognized by CYP11A that only acts on its precursor, vitamin D itself[69]. This likely results in 30 times lower concentration of $20(OH)D_3$ in serum in comparison to $25(OH)D_3$[14]. However, its levels are higher than that of $25(OH)D_3$ in the

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epidermis, a peripheral site of vitamin D3 activation[14]. Therefore, adequate systemic (adrenal gland) or local (immune system) production of CYP11A1-derived vitamin D hydroxyderivatives would require parenteral delivery of vitamin D. These routes of vitamin D precursor delivery could include sublingual tablets, intra-muscular, subcutaneous or intravenous injections as well as its aerosolized form of delivery to the lung (Fig. 1C). As relates CYP11A1-derived products these would be predominantly generated in the adrenal gland for systemic purposes. However, they can also be generated in peripheral organs expressing CYP11A1 including skin and immune system[17,70].

Since vitamin D is readily available, easy to use and relatively nontoxic, it can represent an immediate solution to the problems at relatively high doses, since populations most vulnerable to negative outcome of COVID-19 disease are likely vitamin D deficient and the patients are already under supervision in the hospital environment and are monitored for adverse effects. Vitamin D toxicity is typically not observed until extremely high doses of vitamin D in the range of 50,000-100,000 IUs daily for several months or years[71]. Doses up to 500,000 IUs have been routinely given to nursing home patients twice a year in Scandinavian countries to reduce risk for fracture without any evidence of vitamin D intoxication including hypercalcemia, hyperphosphatemia and soft tissue calcification[71].

In addition, we believe that routes of delivery are likely to impact the final outcome, because bypassing liver vitamin D3 will also be accessible to CYP11A1 for metabolism in organs expressing this enzyme.

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Authors contribution

RMS, CR, ATS: designed figures and concept and wrote the paper

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JS: performed bioinformatics analysis and prepared table 1 and supplemental table 1

MFH, MA and AMJ: wrote the paper

Conflict of interest

The authors declare no conflict of interest

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Figures Legend

Figure 1. Vitamin D as the solution to the COVID-19 illness.

A. Hydroxy-derivatives of vitamin D_3, by inhibition of cytokine storm and oxidative stress, will attenuate ARDS and multiorgan failure induced by COVID-19 .

B. Mechanism of action of canonical and non-canonical vitamin D-hydroxyderivatives. Vitamin D signaling in mononuclear cells downregulates inflammatory genes and suppresses oxidative stress. VDR- vitamin D receptor; RXR- retinoid X receptor; ROR – retinoic acid orphan receptor, RORE- retinoid orphan response element; ARE- antioxidant response element; VDRE- vitamin D response element; Nrf2 - transcription factor NF-E2- related factor 2.

C. Different routes of vitamin D delivery will impact vitamin D activation pattern.

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Table 1. Gene Set Enrichment Analysis (GSEA) of the microarray data deposited at NCBI GEO (GSE117351)).*NES – Normalized Enriched Score; †FDR – False Discovery Rate; (¨) – the effect is absent

ANTIVIRAL PROPERTIES OF VITAMIN D3-HYDROXYDERIVATIVES								
	GSEA for 20,23(OH)$_2$D3				GSEA for 1,25(OH)$_2$D3			
Reactome Pathway	**NES***	**P-value**	**FDR†**	**Direction**	**NES**	**P-value**	**FDR**	**Direction**
Viral mRNA Translation	-2.818	0.00	0.012	Down	-3.601	0.00	0.00	Down
Viral Messenger RNA Synthesis	-2.513	0.00	0.013	Down	-2.405	0.00	1.860	Down
Influenza Infection	-3.171	0.00	3.907	Down	¨	¨	¨	¨
Influenza Viral RNA Transcription & Replication	-3.206	0.00	3.434	Down	¨	¨	¨	¨
Host Interactions with Influenza Factors	-2.249	0.00	0.018	Down	¨	¨	¨	¨
HIV Life Cycle	-2.070	0.00	0.023	Down	-2.503	0.00	7.788	Down
Late Phase of HIV Life Cycle	¨	¨	¨	¨	-2.658	0.00	2.614	Down
Host Interactions with HIV factors	-3.340	0.00	1.354	Down	¨	¨	¨	¨

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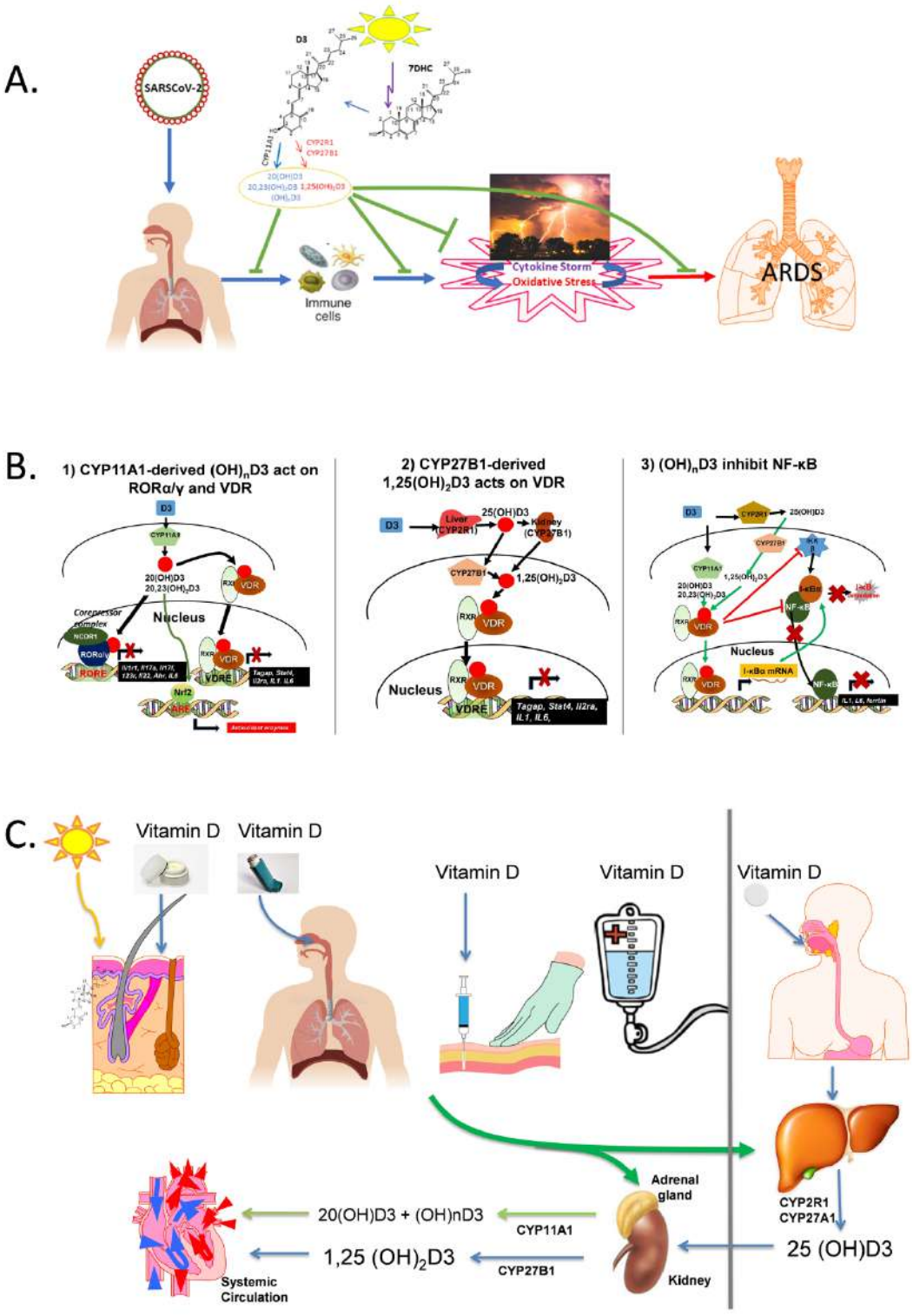

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