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WORD COUNT 2139
TIME SUBMITTED 22-AUG-2022 04:53PM
PAPER ID 89743533

Name of Journal: *World Journal of Clinical Cases*
Manuscript NO: 77544
Manuscript Type: OPINION REVIEW

Combined use of lactoferrin and vitamin D as a preventive and therapeutic supplement for SARS-CoV-2 infection: Current evidence

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Abstract

Lactoferrin is a multifunctional protein that exhibits anti-inflammatory, immune regulating and anti-infective properties. One of its receptor sites is located on severe acute respiratory syndrome coronavirus 2. The binding of lactoferrin with heparin sulfate proteoglycans may prevent the first contact between the virus and host cells, thus preventing subsequent infection. Given that lactoferrin may act as a natural mucosal barrier, an intranasal treatment together with its oral intake can be hypothesized to prevent the spread, infection and inflammation caused by coronavirus disease 2019 (COVID-19). Moreover, the literature reports that vitamin D plays an essential role in promoting immune response. With its anti-inflammatory and immunoregulatory properties, vitamin D is critical for activating the immune system's defenses, improving immune cell function. Different studies also demonstrate that lactoferrin is a potential activator of the vitamin D receptor. In this sense, the combined use of lactoferrin (through an association of oral intake and a nasal spray formulation) and vitamin D could represent a valuable therapy for COVID-19 treatment and prevention. However, further randomized clinical trials are needed before recommending/prescribing them.

INTRODUCTION

Lactoferrin (LF), which belongs to the genus of glycoproteins and is contained in human secretions, is part of a non-specific defense system with a significant role in fighting both bacterial and viral infections, in addition to producing significant anti-inflammatory effects on various mucosal surfaces and acting in the regulation of iron metabolism[1]. However, it is worth noting that the biological activities of LF have been attributed only in part to the iron-sequestering activity[2], given that its mechanism of action also involves binding to other specific receptors, cell signaling and protein folding[3,4]. An important study reported that LF can directly interact with virus capsid proteins[5], which may hinder viral entry into target cells by blocking the virus from binding to host cell components that are used as receptors or co-receptors.

Direct binding of LF to viral particles has been recognized in the literature for many viruses[6-9]. In this sense, a protective role of LF could also occur in relation to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection[10,11]. In this review, we discuss evidence on the potential clinical and molecular effects of LF (alone or in combination with another supplement) related to coronavirus disease 2019 (COVID-19) prevention or treatment.

SCIENTIFIC FINDINGS SUPPORTING THE ROLE OF LACTOFERRIN IN SARS-COV-2 INFECTION

In the current scientific landscape, special attention has been paid to the potential role of LF as a supplemental adjuvant, whether in terms of preventing SARS-CoV-2 infection or treating COVID-19, due to its ability to interact with different receptors. Results of recent research show that the cellular entry of the virus occurs via high-affinity interactions between the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and the human host angiotensin-converting enzyme 2 (ACE2) receptor[12]. However, for cellular entry of the virus, there must also be the interaction of SARS-CoV-2 with other molecules, including heparan sulfate and cell proteases (such as lysosome-localized Cathepsin B/L and serine proteases of the TMPRSS family). This is essential to virus adhesion to the cell membrane, which may facilitate the interaction of

the viral spike protein with the ACE-2 receptor, as well as the internalization process of SARS-CoV-2[13-15].

In particular the binding of LF with heparin sulfate proteoglycans (HSPG) may prevent the first contact between the virus and host cells, thus preventing subsequent infection. Indeed, in SARS-CoV-2 infections, HSPGs play a considerable role in the cell entry process, as the anchoring sites provided by HSPGs allow the initial interaction between SARS-CoV-2 and host cells and the concentration of viral particles on the cell surface. In other words, the virus, by binding to HSPGs, rolls across the cell membrane and scans specific entry receptors, thus enhancing the ability of the virus to infect the host cell[16].

Computational studies[17] have sought explanations for a direct interaction between LF and the ACE2 receptor or between LF and the viral spike protein, identifying possible reciprocal interactions that may provide a molecular explanation for the preventive effect of LF against SARS-CoV-2 infection. The results found show that LF has the ability to bind to the ectodomain of ACE2 with a significantly high affinity. On the other hand, the researchers did not observe any binding to RBD up to the maximum "physiological" concentration range of LF. This suggests that the inhibitory effect of LF on the formation of the ACE2/RBD complex may be related to its binding to the ACE2 receptor, thus weakening the evidence of binding to the RBD domain of the spike protein[18].

Based on data from the animal and human studies, Zimecki *et al*[19] postulated that LF may have a clinical benefit in preventing and ameliorating the COVID-19 induced cytokine storm and its devastating consequences on lungs and other vital organs. Kell *et al*[20] suggest a similar process. More precisely, LF is capable of performing two functions in cases of SARS-CoV-2 infection: on the one hand, it sequesters iron and inflammatory molecules that significantly increase during the cytokine storm; on the other hand, it helps to occupy receptors (ACE2/RBD) and HSPGs, preventing the virus from binding to host cells. Receptor blockade is, in fact, an important peculiarity of LF,

when used as a supplement. Moreover, LF may help prevent thrombocytopenia and hypercoagulation, which are major complications of COVID-19.

Studies with swabs for SARS-CoV-2 detection demonstrated that viral RNA in the upper airways has higher viral loads than in the throat, in both symptomatic and asymptomatic patients. This suggests that the nasal epithelium may be an important site for initial infection, acting as a "key reservoir" for viral spread through the respiratory mucosa-[12]. In light of this, it could be hypothesized that local treatment of the nasal mucosa with LF in a nasal spray formulation or through its oral intake may counteract SARS-CoV-2 infection and inflammation. LF, in fact, has the ability to serve as a natural barrier of the respiratory and intestinal mucosa, and, moreover, its inclusion in preservative structures (liposomes) may reduce gastric and intestinal denaturation, which is critical to maintaining its integrity and biological functionality. Such findings suggest that LF could be a supplement to be used in both asymptomatic and mildly symptomatic patients to prevent the exacerbation of COVID-19[21].

MYELOID-DERIVED SUPPRESSOR CELLS VERSUS COVID-19: ACTION OF LACTOFERRIN

Recent research has verified the presence of a dysregulated myeloid cell compartment containing an increased number of myeloid-derived suppressor cells (MDSCs) in severe COVID-19 patients, which may be correlated with disease severity. In this sense, the mechanism of action of MDSCs involves promoting the survival of SARS-CoV-2 through the suppression of T-cell responses, leading to a highly pro-inflammatory state in response to the secretion of various mediators of immune activation[22]. MDSCs are defined as bone marrow-derived innate immune cells with ability to suppress effector T-cell responses. This heterogeneous population of cells is composed mainly of two distinct subtypes that include polymorphonuclear or granulocytic-MDSCs (PMN-/G-MDSCs) and monocytic MDSCs (M-MDSCs). Among their roles is the ability to regulate a wide variety of adaptive (T and B cells) and innate (including natural killer cells, macrophages, and dendritic cells) immune cells[23].

Results of an important study that evaluated the effect of LF on MDSCs show that MDSCs from mice and human infants are sensitive to LF, whereas the same is not true of adult MDSCs. To explain the rationale for this reduced sensitivity, the authors also assessed the expression of different receptors capable of binding to LF, such as lipoprotein receptor-related protein (LRP)-1, LRP2, intelectin (ITLN)-1 and ITLN2. MDSCs from newborn mice were found to have a much stronger expression of LRP2 compared to ITLN1, ITLNb or LRP1. Another finding of interest was that the expression of LRP2 decreased with age, resulting in a substantial reduction in LRP2 expression on the cell surface[24]. This may, in addition to explaining differences in the sensitivity of MDSCs to LF between infants and adults, serve as evidence to understand more about the molecular peculiarities of COVID-19 in different age groups in order to propose more appropriate preventive and/or therapeutic protocols.

EVIDENCE FOR COMBINED USE OF LACTOFERRIN AND VITAMIN D

In light of the benefits that seem to be associated with the use of LF in COVID-19 patients, it may be useful to conduct further studies on the potential benefits of a combination of LF with others nutraceuticals in order to verify the possible synergistic effects in the fight against SARS-CoV-2 infection. In particular in recent years, research on vitamin D has been extensive and evolving, evidencing that there is an important interaction between vitamin D and cells of the innate and adaptive immune system. Regarding the role in supporting innate immunity, it is known that serum 25-hydroxyvitamin D [25-(OH)D] bound to vitamin D-binding protein allows intracellular entry of free [25-(OH)D] into antigen-presenting cells (APCs). This results in both endogenous production and the action of 1-alpha, 25-dihydroxyvitamin D [1,25(OH)2D] through the vitamin D receptor (VDR), leading to the induction of antimicrobial proteins like cathelicidin, nuclear factor kappa β, and β-defensins that may contribute to the elimination of SARS-CoV-2[26].

In this context, the innate immune system is expected to fight the viral infection first until the adaptive immune system (T and B cells) is sufficiently activated, which

usually occurs within 7 to 10 d after the primary infection. Moreover, SARS-CoV-2 infection causes the activation of APCs that can induce SARS-CoV-2 phagocytosis, through communication with naïve T cells (Th-0). An optimal serum vitamin D level may lead to differentiation of naïve T cells into T helper (Th) 2 cells instead of Th1 cells, promoting the production of anti-inflammatory cytokines such as interleukin (IL)-10, Il-5, and Il-4. Anti-inflammatory cytokines have the ability to decrease the secretion of pro-inflammatory cytokines, including interferon-γ, IL-6, IL-2 and tumor necrosis factor-α, by down-regulation of Th1 cells. All this may result in an important anti-inflammatory reaction with potential to control the overreaction of the immune system against COVID-19[25].

On the other hand, in the presence of vitamin D deficiency, the adaptive immune response shifts towards differentiation of naïve T cells into Th1 cells, which may cause hyper-inflammation/cytokine storm[25]. According to Prietl *et al*[26], numerous cells (including immune cells) possess enzymes that metabolize vitamin D. This process appears to be critical for normal immune function; indeed, altered or insufficient levels of vitamin D may cause dysregulation of immune responses. In an important review article, four potential mechanisms by which vitamin D may affect T-cell function were proposed: direct endocrine effects on T lymphocytes mediated by systemic calcitriol; direct intracrine conversion of 25(OH)D to calcitriol by T lymphocytes; direct paracrine effects of calcitriol on T cells following conversion of 25(OH)D to calcitriol by monocytes or dendritic cells; or indirect effects on antigen presentation to T lymphocytes mediated by localized antigen presenting cells (APCs) affected by calcitriol[27].

Two recent studies have evaluated the effects of LF on vitamin D. The first demonstrated that LF is a potential activator of the vitamin D receptor (VDR) through the expression of VDR messenger ribonucleic acid (mRNA), postulating that LF directly targets the cell nucleus to regulate VDR transcription activity in vitamin D-deficient mice. LF appears to show an affinity for three specific deoxyribonucleic acid (DNA) sequences that interestingly appear similar in the VDR- promoter. Thus, the authors of

this study hypothesize that LF regulates VDR expression by binding directly to these DNA elements and that therefore it may act as a transcription factor or coactivator to stimulate VDR expression[28]. In the other study, Wang *et al*[29] demonstrated that LF, in addition to having a protective role in the intestine in mice deficient in vitamin D and reducing elevated serum pro-inflammatory cytokines, it also has the ability to stimulate, increasing the expression of the vitamin D receptor.

CONCLUSION

The combined use of LF and vitamin D could represent, through a synergistic action, a valuable therapeutic support and also for the prevention of SARS-CoV-2 infection. In addition, the association of oral intake of LF and a nasal spray formulation would be an additional tool to prevent the spread and worsening of the infection. Although the combined use of LF and vitamin D seems to be a promising approach as an adjuvant for the COVID-19 management, there are still no *in vivo* studies with robust evidence to prove the benefits of using this combination of supplements against SARS-CoV-2 infection. Further randomized clinical trials are needed to evidence any related beneficial action before recommending/prescribing them for the population[30]. With the exception of the use of approved drugs, what is known so far is that the practice of physical exercises may be an ally for COVID-19 prevention and treatment, as well as to enhance SARS-CoV-2 vaccine immunogenicity[31].

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