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Defensins: Therapeutic molecules with potential to treat SARS-CoV-2 infection

The coronavirus disease-19 (COVID-19) pandemic caused by SARS-CoV-2[1], has led to unprecedented health crisis globally. To date, there is no effective drug that is specific towards the SARS-CoV-2 virus. Hence, it is of considerable interest to explore whether defensins, crucial components of host-defense in humans[2,3], can be developed as therapeutic molecules. Defensins are cationic peptides with three disulphide bonds and are the first line of defense against bacteria and fungi in mammals. They are classified as α- or β-defensins based on their disulphide connectivity. These peptides interact with microbes, causing membrane permeation, electrochemical gradient disruption and inhibit metabolic processes[2,3]. Defensins have wide cellular distribution, being present in airway epithelium, intestinal epithelium and skin[2,3]. α-Defensins are mainly expressed in neutrophils and paneth cells in the intestine. β-Defensins are found primarily in epithelial cells, including oral, airway and skin epithelium, and play a crucial and important role in pulmonary defense against viruses[4]. Their expression is mediated by toll-like receptors (TLRs). β-Defensins serve as ligands for TLRs[5].

There has been renewed interest in host-defense peptides, particularly LL-37, the C-terminal segment of human cathelicidin antimicrobial peptide[6] and defensins due to the current COVID-19 pandemic and their antiviral activities on other viruses reported earlier[4,6]. LL-37 and defensins have the ability to kill respiratory viruses under *in vitro* conditions[4,6]. In humans, the defensin family of peptides identified in neutrophils was classified as human neutrophil peptides (HNPs)[1,3,7]. There are six HNPs. Of the four HNPs, HNP1 to HNP3 occur at relatively high concentrations as compared to HNP4[3,8,9]; human α-defensins 5 and 6 (HD5 and HD6) are found in paneth cells and are involved in gut innate immunity[8]. HNPs have antimicrobial activity against Gram-positive and Gram-negative bacteria[1,9]. The antiviral activity of HNP1 to HNP3 and HD5 was observed in SARS-CoV-2 infection, but HNP4 activity against virus was low[10] probably due to its presence in low abundance. HD5 and HNP1 inhibited viral infection in the Gamma and Alpha variants, suggesting that healthy neutrophils counteract virus infection through innate immunity[10]. HD5 prevents SARS-CoV-2 infection by binding to the host's angiotensin-converting enzyme 2 (ACE2), which is the receptor for the virus[11]. *In silico* approaches also suggest that Anti microbial peptides (AMPs) have the ability to interact with spike surface viral protein and thereby prevent its binding to ACE2[12].

Human β-defensins (HBDs) have antiviral activity *in vitro* against both enveloped and non-enveloped viruses[13,14]. The antiviral activity of HBDs and their variants against HIV, respiratory syncytial virus (RSV), human papillomavirus, herpes simplex virus, Zika virus and Middle East respiratory syndrome coronavirus (MERS-CoV) has been extensively studied[14-18]. HBD2 and HBD3 were observed to suppress HIV infection[17,18]. In HIV-positive individuals, the expression of HBD2 was diminished considerably[18]. The expression of HBD2 throughout the respiratory epithelium and its suppression on viral infection[14-18] suggests that it plays a role in respiratory diseases. Antiviral activity of HBD2 was observed specifically in respiratory infections such as Influenza virus, RSV and Rhinovirus infection[19,20]. In influenza A virus infection, increased expression of HBD2 was found in the respiratory track both *in vitro* and *in vivo*. In a murine model, inhibitory activity of recombinant mouse defensins mBD2 and mBD3 against enveloped influenza A virus was observed[19]. In contrast, downregulation of chinchilla defensin cBD1 observed against RSV infection in chinchillas resulted in bacterial colonization in the nasopharynx[21]. In A549 human alveolar type II-like epithelial cells, RSV and adenovirus infection induced production of HBD2 which was blocked with recombinant HBD2 treatment[22]. It is evident that defensins are associated

with viral infections notwithstanding their complex regulation on infection. Hence, for SARS-CoV-2 infection, it would be pertinent to explore the development of defensins as therapeutic agents to treat COVID-19.

SARS-CoV-2 is a single-stranded enveloped virus[23]. It has 79 per cent genome similarity to SARS-CoV-1 and 50 per cent genome similarity to MERS-CoV. The SARS-CoV-2 virus encompasses the replicase protein (ORF), spike protein (S), envelop protein (E), membrane protein (M), and nucleocapsid (N) structural protein. This S protein aids in entry of the virus into the host cell by binding through the receptor-binding domain (RBD) to ACE2, on the cell surface. Along with transmembrane enzyme serine protease 2, furin, and cathepsin L, proteolytic cleavage of the S protein activates viral entry[23]. Interaction between HD5 and ACE2 studied by molecular docking and biophysical methods indicate that HD5 binds to ACE2 receptor[10,24]. Defensins could conceivably target the spike protein receptor, ACE2, spike protein or disrupt the viral membrane.

In our previous study, it was observed that the β-defensin genes *HBD2*, *HBD6*, *HBD7* and *HBD3* were found significantly downregulated in nasopharyngeal/oropharyngeal samples of patients infected with SARS-CoV-2. This suggests a connection between defensins and SARS-CoV-2 infection[25]. A non-significant downregulation of α-defensin genes, such as *DEFA3* and *DEFA6* was observed during the SARS-CoV-2 infection[24]. Association of defensin genes with SARS-CoV-2 infection suggested that upregulating their gene expression could be an attractive therapeutic intervention. HBD2 conjugated-MERS RBD was found to enhance primary innate immune response followed by effective adaptive immune response[11,26]. P9, a mouse β-defensin 4 peptide, showed activity against MERS-CoV and SARS-CoV-2 viruses[4,15]. Using *in silico* methods, HBD3 conjugated to B-cell, helper T-lymphocyte and cytotoxic T-lymphocyte elicited a robust immune response[4]. Defensins HBD2 and HBD3 were also used as adjuvants to design vaccines against MERS-CoV. HBD2 conjugated to MERS-CoV-S-RBD elicited a much better immune response as compared to S protein RBD alone, in mouse models[4].

There are several antiviral drugs that have been re-purposed to treat SARS-CoV-2 infection. These include anti-malarial drugs chloroquine and hydroxychloroquine, anti-HIV drug lopinavir/ritonavir; remdesivir used as antiviral drug against Ebola virus, favipiravir, ribavirin, azithromycin, umifenovir and oseltamivir have been used for SARS-CoV-2 treatment[27]. However, these are not specific to SARS-CoV-2 and their routine use to treat COVID-19 is controversial. Small molecule drugs molnupiravir and paxlovid appear to be effective in reducing hospitalization and mortality[28]. However, detailed results of clinical trials have not yet been published.

AMPs including defensins[29] have not been popular as drugs due to their susceptibility to enzymatic degradation. However, their ability to combat viral infections has been extensively studied. The U.S. Food and Drug Administration has approved a few AMPs, including gramicidin, daptomycin and vancomycin, which are used as antibacterial drugs[30,31]. Several antimicrobial peptides are in various stages of clinical trials[30,31].

In summary, defensins exhibit potent antiviral activity against several viruses *in vitro* and possibly are the first line of defense against viruses, as in the case of bacteria, *in vivo* also. Although human defensins were first characterized as antimicrobial peptides, subsequent investigations have indicated that these have several biological activities such as modulation of the immune system, lyse cancer cells, wound repair and act as chemoattractants[3,8]. The downregulation of β-defensin genes on infection with SARS-Cov2 and the *in vitro* antiviral activity of α-defensins against SARS-CoV-2 suggests that these peptides have a role in defense against SARS-CoV-2 and possibly other viruses that are responsible for upper respiratory tract infections. Developing suitable formulations of these peptides would be necessary for therapeutic applications as these could be susceptible to proteolytic degradation *in vivo*. These peptides exhibit other biological activities apart from antibacterial activity. Hence, their dosage would have to be adjusted for antiviral activity without any deleterious side effects. A nasal spray formulation would be of interest for delivery at the initial site of infection rather than systemic administration.

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