Milk-Derived Antiviral Peptides Targeting Zoonotic Viruses

Subjects: Others

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Animals often serve as reservoirs for viral zoonoses, diseases transmissible from animals to humans. While zoonotic viral diseases have been present in human populations since the inception of agricultural practices, they have gained increasing prominence as a global public health concern, particularly with recent epidemics like SARS-CoV-2 (COVID-19). Some of these diseases are categorized as “emerging infectious diseases” due to their newfound recognition or significant changes in their range and epidemiology. Notable zoonotic diseases include influenza, Ebola virus, West Nile virus, emerging coronaviruses, monkeypox, rabies, Zika, and Lyme disease. Six out of every ten infectious diseases in humans are zoonotic, with many being viral. Therefore, it is imperative to enhance our capabilities to prevent and respond to these diseases, adopting a One Health approach. Finding new therapies and ways to prevent viral zoonoses is just as important as increasing efforts on surveillance and early detection, and with the rising amount of research demonstrating the potential of bioactive peptides produced from milk as antivirals, a vital opportunity arises to assess their usage in viral diseases with a focus on One Health. Indeed, several peptides derived from milk protein parents have shown potential for zoonotic viral diseases.

Keywords: milk ; peptides ; zoonoses ; antiviral ; one health

1. COVID-19

Severe acute respiratory syndrome (SARS) is a viral respiratory disease induced by SARS-associated coronavirus (SARS-CoV) which affected Asia, North America, and Europe in 2002–2003 [1]. In late 2019, a new type of coronavirus called SARS-CoV-2 caused a viral pandemic known as Coronavirus disease (COVID-19), which led to severe respiratory problems. Worldwide, researchers searched earnestly for molecules that could help create therapies for COVID-19 prevention and treatment. Coronaviruses enter target cells by fusing the membranes of the virus and the host cell, which is mediated by a viral spike glycoprotein (S protein) [2]. Earlier in 2020 a study showed that whey proteins from human, goat, and cow milk could inhibit SARS-CoV-2 entry and replication in Vero E6 and A549 cell lines, with an EC_{50} of about 0.13 mg/mL of total protein [3]. Among these molecules, milk and whey proteins, particularly lactoferrin, have been identified as one of the main antivirals present in milk and have been reviewed [4]. Lactoferrin was shown to present an array of activities against the virus, including a high affinity with the spike domain [5], competing for binding to an ACE2 receptor [6], and blocking the spike protein Furin-cleavage site [7]. It also hinders viral attachment by binding at the level of heparan sulfate proteoglycans [8][9] and binding to sialic acid [6]. Lactoferrin also reduces SARS-CoV-2 infectivity by inhibiting cathepsin L activity [10] and acts as an immune modulator of the antiviral immune response [11]. Lactoferrin was also shown to potentiate the effect of certain treatments towards SARS-CoV-2, such as remdesivir, hypothiocyanite anion, and the oral administration of liposomal Lf and oral zinc solution [9][12][13][14][15]. In addition to lactoferrin, other milk proteins were demonstrated to present antiviral activity against SARS-CoV-2 [16]; in particular, two β-lactoglobulin-derived peptides, produced by trypsin digestion, Ala-Leu-Pro-Met-His-Ile-Arg (ALMPHIR) and Ile-Pro-Ala-Val-Phe-Lys (IPAVFK), exhibited the ability to inactivate the virus via cathepsin inhibition and binding to the spike protein and also the host cell membrane receptor, showing great potential as a treatment for SARS-CoV-2 [10][17].

Interestingly, recent works have shown that milk-derived peptides are effective multi-targeted therapeutic candidates to treat SARS-CoV-2. Pradeep et al. [16] showed an interesting strategy involving the concurrent blockade of diverse pathways in the infectious cycle of SARS-CoV-2 to mitigate the COVID-19 threat. Through a combination of molecular docking, molecular simulation, heat mapping, and manual interpretation, the study developed a new strategy to identify a plethora of peptides capable of impeding the spread of the coronavirus [16].

2. Human Immunodeficiency Virus (HIV)

Human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS) are caused by a retrovirus known as the human immunodeficiency virus (HIV). HIV is a life-threatening infection that has spread globally since the first case was discovered, leading to a significant need for effective antiviral therapies. Milk-derived proteins such as human and bovine lactoferrin, modified β-lactoglobulin, α-lactalbumin, and lactoperoxidase have all demonstrated antiviral properties against human immunodeficiency viruses, mostly via the ability to link themselves to host cell receptors, inhibiting not only viral absorption but also the virus replication cycle [18][19][20][21][22][23].

Bovine lactoferrin and its derived peptide lactoferricin inhibit HIV-1 by acting on CXCR4 and CCR5 receptors [18][23]. Recently Berkhout et al. [18] showed that lactoferricin presents a lower inhibition when compared to lactoferrin, suggesting that other domains in the native protein may also aid in inhibition. Indeed, the apo form of bovine lactoferrin also inhibits HIV-1 replication in a different study [24]. Other whey proteins were also shown to inhibit the HIV virus. Modified β-lactoglobulin and α-lactalbumin were able to block the virus entry in the host cells due to interactions with the gp120 envelope protein [25][26], and Tenascin-C also displayed antiviral activity against HIV by interacting with its envelope domain, effectively neutralizing the retrovirus, and preventing transmission via breast milk [27]. In fact, the potency of Tenascin-C's inhibition surpasses that of lactoferrin and is comparable to HIV-1-neutralizing monoclonal antibodies [28].

3. Human Cytomegalovirus (HCMV)

Human cytomegalovirus (HCMV), also known as human herpesvirus 5 (HHV-5), is a virus that belongs to the *Herpesviridae* family. This virus can enter the human body through contact with mucous membranes or through blood components containing cells, as well as through stem cell/organ transplants [29]. In human cytomegalovirus infection, lactoferrin, lactoferricin, and methylated β-lactoglobulin and α-lactalbumin can inhibit virus replication and transcription by interacting with the viral genome [30][31]. Lactoferrin and lactoferricin display different mechanisms of action, being able to both interfere with the virus target cells and up-regulate the immune system, but also exerting a synergistic antiviral effect with cidofovir, an antiviral drug commonly used in patients with human cytomegalovirus [32][33][34][35][36][37]. Methylated β-lactoglobulin and α-lactalbumin, on the other hand, inhibit viral replication and transcription by interacting with the viral genome [4][30].

4. Hepatitis B

Hepatitis B is an often-life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem that can cause chronic infection and puts people at great risk of death from cirrhosis and liver cancer. The inhibition of hepatitis B (and C) viruses was shown by human and bovine lactoferrin (and peptide derivatives) as well as by α-lactalbumin, β-lactoglobulin, and lysozyme, via interaction between viral and cell proteins that interfere with virus entry and multiplication [38]. Here, lactoferrin, when saturated with zinc or iron, showed antiviral activity against this disease. As for the mechanism of action, lactoferrin, saturated with the positive ions mentioned above, was able to bind to several molecules of the host cell and, therefore, interfere with the virus's ability to attach itself to the host cell and its ability to enter the host cell [39][40].

5. Influenza

Influenza viruses seriously threaten global health, causing substantial morbidity and mortality, especially among individuals with weakened immune systems [41]. Whilst vaccination remains a cornerstone for infection control, its effectiveness is compromised by the rapid antigenic drift and the emergence of new viral subtypes [41]. Breastfeeding has been acknowledged for its protective role against respiratory and gastrointestinal infections in infants, so it would be expected that milk-derived peptides exert some activity against this virus [42]. Indeed, an early study found that lactoferrin exerts a protective effect against influenza-induced apoptosis by modulating caspase 3 function and impeding the export of viral ribonucleoproteins from the nucleus to the cytoplasm [43]. Subsequent research revealed a lactoferrin-derived peptide, bLf, which was shown to bind to the viral HA, inhibiting both hemagglutination and infection by influenza A viruses, of both group 1 and group 2 subtypes. Notably, bLf demonstrated binding to the HA2 subunit, which harbors the universally conserved HA epitope, explaining the broad-spectrum anti-influenza activity observed [41]. In a more recent study, Scala et al. [44] delved deeper into the inhibitory potential of lactoferrin-derived peptides against influenza virus infection and identified novel sequences derived from the C-lobe of bovine lactoferrin, demonstrating broad anti-influenza activity and the ability to prevent viral hemagglutination and infection at remarkably low concentrations [44]. Some in vitro studies have reported that lactoferrin and lactoperoxidase may also depend on other mechanisms, such as the inhibition of viral shedding, leading to the suppression of influenza virus A (H1N1) [45][46]. Bovine lactoferrin was found to interact

with viral haemagglutinin, which resulted in the inhibition of virus-induced haemagglutination for influenza A virus. [41]; a similar mechanism of action was observed for glycomacropeptide in previous studies on influenza virus A [47].

6. Zika and Usutu Virus

Zika virus is a mosquito-borne flavivirus that gained global attention due to its association with serious health concerns. Infection with Zika virus has been linked to neurological complications, including microcephaly in infants born to infected mothers, making it a significant public health issue [48]. A recent study [49] explored the antiviral properties of human milk at different stages of maturation against Zika virus and Usutu virus. The results indicated that human milk exhibited antiviral activity against both Zika virus and Usutu virus across all stages of lactation, and that extracellular vesicles and glycosaminoglycans played a role in the protective effect of milk, with no significant variations observed between colostrum, transitional, or mature milk. Mechanistic studies revealed that the mechanism was not due to the inactivation of the viral particles but was instead due to blocking the binding of both flaviviruses to cells.

7. Rotavirus

Rotavirus is a global pathogen that is the major cause of severe diarrhea in infant mammals. In vitro tests showed the ability of human milk fractions to inhibit rotavirus replication, [50] particularly a mucin complex fraction containing the milk-fat globule membrane proteins MUC, lactadherin, and an unidentified 80-kDa whey protein [51][52]. While it was suggested that lactadherin might be responsible for the action of the mucin complex [51][52], MUC1 showed antiviral activity by inhibiting the replication of 3 human rotavirus strains [50]. Interestingly, only the human form of lactadherin could inhibit Wa rotavirus infection in vitro, apparently through a mechanism involving protein–virus interactions, which is dependent on the protein structure or the attached oligosaccharides [53]. According to Yolken et al. [51], the sialic acid present in lactadherin also plays a vital role in its antiviral action. Also, several whey proteins, including apo-lactoferrin (iron-free), homo-lactoferrin (carrying Fe3+), α-lactalbumin, and β-lactoglobulin, have demonstrated the capacity to hinder the attachment of rotavirus viral particles to host cellular receptors [54].

8. Dengue Virus

Dengue is a significant mosquito-borne viral disease in tropical and subtropical regions. The responsible pathogen, dengue virus (DENV), consists of four distinctive serotypes: DENV-1, -2, -3, and -4. The infection can be mild or can result in clinically severe presentations, causing mild dengue fever, the more serious dengue hemorrhagic fever, or dengue shock syndrome [55]. A study in 2017 [56] reported the antiviral effect of bovine lactoferrin against DENV infection both in vivo and in vitro. Lactoferrin significantly inhibited the infection of the four serotypes of DENV and blocked binding between DENV-2 and the cellular membrane by interacting with heparan sulfate (HS), dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), and low-density lipoprotein receptors (LDLR) [56].

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