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Review Article

Camel Milk lactoferrin: Special agent against bacterial infections

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ABSTRACT

Actually milk lactoferrin is one multifunctional protein topromote bacterial clearance. Lactoferrin is one glycoproteins detected in the livestock milk; as camel milk containing highest amount in compared to other livestock species. Lactoferrin boosts the immune system by protecting the cells against bacterial and viral infections and inflammations. Probably the main physiological function of lactoferrin as antibacterial agent is binding to the iron, and interaction with different cellular receptors, could be great reason for the antimicrobial activity. According to studies, iron withholding capacity of lactoferrin influences the activation of immune cells and inhibits biofilm formation of pathogenic microorganism. All bacteria require iron for growth and their virulence is related to iron availability. Iron limitation in mucosal secretions, as first defense line against microorganisms hinders bacterial growth. Biofilm formation is major agent for virulence of bacteria. Lactoferrin can reduce bacterial growth, inhibit bacterial adhesion and biofilm formation; thus, it might be considered as antimicrobial therapeutic agent. Regarding the increasing resistance to antibiotics, it is necessary to explore novel antimicrobial drugs to bacterial diseases.

Key words: Camel Milk lactoferrin, anti bacterial, infections

INTRODUCTION

Actually lactoferrin works as an opsonin to induce bacterial clearance. In addition to iron, lactoferrin can able to bind other compounds such as lipopolysaccharide, heparin, glycosaminoglycan's, DNA, or ions such as Ga^{3+}, Mn^{3+}, Cu^{2+} and Zn^{2+}. Probably the main physiological function of lactoferrin as antibacterial agent is binding to the iron, and or sequestering iron as a necessary requirement for most bacterial pathogens. Thus growth of a broad range of bacterial strains will be inhibited (Janssen and Hancock, 2009).

Bacteriostatic function of lactoferrin is due to bind the Fe^{3+} ion and limiting Fe^{3+} for bacteria growth and their virulence at the infection site, motility and biofilm formation of pathogenic bacteria will be inhibited (Gonzalez-Chavez et al., 2009). Lactoferrin has bactericidal action due to some

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reasons such as direct interaction with lipopolysaccharides of bacterial surfaces, damages membrane of Gram-negative bacteria, increase the membrane's permeability, and enhances lysozyme action and antibiotics drugs (Gonzalez-Chavez et al., 2009). Lactoferrin effects against Gram-positive bacteria are due to binding to anionic molecules such as lipoteichoic acid and prevent the attachment of these bacteria to the host cell surfaces (Leitch and Wilcox 1999). So lactoferrin and lysozyme exert combined effect against Gram-positive and negative bacteria (Quiroz et al., 2013).

The effect of milk lactoferrin against pathogenic bacteria: The antibacterial activity is the first biological function of lactoferrin in host pre-immune defense system. The lactoferrin of mammalian species have been proved to inhibit the growth of some pathogenic strains in human and/or animal such as *Escherichia coli, Salmonella typhimurium, Shigella dysenteriae, Listeria monocytogenes, Streptococcus* spp., *Vibrio cholerae, Legionella pneumophila, Klebsiella pneumophila, Enterococcus* spp., *Staphylococcus* spp., *Bacillus stearothermophilus* and *Bacillus subtilis* (Valenti and Antonini, 2005).

Breifly the action mechanism of lactoferiin on different types of bacteria summarized according to Embelton et al (2013). Destabilization of micro-organism membrane; for Gram-negatives, lactoferrin binds to porins present on the cell surface, release lipopolysaccharides, and increase bacterial membrane permeability (Valenti and Antonini, 2005). Also binding of lactoferrin to calcium induces lipopolysaccharides release. The membranes of gram-positive bacteria are disrupted by binding of hydrophobic residues in the N-lobe of lactoferrin to lipothichoic acid (Suzuki et al 2008).

Alterations of micro-organism motility; glycosylated lactoferrin bind to bacterial adhesion sites on bacteria and host cells and prevent bacterial attachment. Lactoferrin binds receptors on host cells such as glycosaminoglycan that are the entry way for viral and bacterial pathogens. Thus lactoferrin by competitive inhibition reduce endocytosis of the micro-organism into host cells. This mechanism is used by some strains of *E. coli* and *Staphylococcus aureus* as entero invasive, even it protects cells against viral infections (Van der Strate et al., 2001).Iron-binding activity of lactoferrin cause to moving of bacteria to find iron, thus the bacterial biofilms will be disrupted. Virulence of *Pseudomonas and Burkholderia spp*., (in cystic fibrosis) is through biofilm formation, and the lactoferrin has protective effect due to iron binding capacity (Van der Strate et al., 2001). Modification of virulence factors; lactoferrin may degrade protein virulence factors of many bacteria through proteolysis. This proteolysis induced by the N-lobe of lactoferrin and it's confirmed for *H. influenza, Shigella spp.* and *E. coli* (Ochoa and Clearly, 2004).

Effects of lactoferrin on benefit bacteria in the gut: It is concluded that the gut protection by the human milk is due the presence of various functional proteins, such as immunoglobulin A (IgA), lactoferrin, growth factors and cytokines (Quiroz et al., 2013). Immuno nutrients in the milk including amino acids, fatty acids, lysozyme, minerals such as zinc, and prebiotic oligosaccharides which play an key role in the maturation and health of the child´s gastrointestinal tract (Embelton et al., 2013). Glutamine and arginine influence gut integrity and vitamins have basic roles in antioxidant protection. Lactoferrin has great importance in the defense line against gastrointestinal diseases (Embelton et al., 2013; Quiroz et al., 2013)

The development of the intestinal micro biota in breast-fed children is quite different from artificial feeding. The intestinal flora pattern of breastfed babies consisting of high percentages of lactobacilli, especially *Lactobacillus bifidus*, while babies fed with cow´s milk or formulas have microbiota similar to the adults (Quiroz et al., 2013).

The human milk have probiotic action and stimulate the growth of beneficial bacteria such as *bifidobacterium* and *lactobacilli*, that protecting the intestine by limiting the different pathogens throuhg decreasing of the intestinal pH (Lönnerdal, 2003).

Oral administration of lactoferrin reduces bacterial infections of the gastrointestinal tract and promoting the proliferation and growth of bacteria with low iron requirements such as Lactobacillus and *Bifidobacteria* as beneficial strains for host (Sherman et al., 2004). But administration of lactoferrin as intra peritoneally, intravenously or intramuscularly is rapidly cleared from the body of experimental animals, that reporting little or no protective effect against bacterial infections (Valenti and Antonini, 2005).

The anti-bacterial mechanisms of milk lactoferrin

Bacteriostatic activity of milk lactoferrin: All bacteria require iron for growth and their virulence is related to iron availability. Iron limitation in mucosal secretions, as first defense line against microorganisms inhibits bacterial growth (Valenti and Antonini, 2005). The lactoferrin found in

secretions is almost iron free and it tightly bind iron Fe3+ (two Fe3+ ions per molecule), with an affinity and stability much higher than transferrin. The presence of apo-Lf in mucosal surfaces maintains the iron level below required level for microbial growth. According to studies iron sequestration by apo-Lf can effectively inhibit the growth of many bacterial species due to iron deprivation and can be completely recovered after iron supplementation (Berlutti et al., 2011).

In addition, most pathogenic bacteria can acquire iron by means of two principal ways: secret small iron chelators or acquiring iron directly from transferrin and lactoferrin. Regarding to the first system, many bacteria synthesize small iron-chelating molecules or siderophores as microbial virulence factors that compete with Lf for insoluble Fe^{3+} ions, which bind Fe^{3+} ions with high affinity and transport it into cells through a specific membrane receptors (Orsi, 2004).

Another mechanism for iron acquisition by pathogenic bacteria is removing of iron from hemin that released from hemoglobin. Although lactoferrin can efficiently compete with bacteria for hemin iron but still many Gram-negative pathogenic bacteria with hemin iron-acquisition systems can acquire iron directly from transferrin and lactoferrin by two different bacterial receptors (Valenti and Antonini, 2005).

Bactericidal activity of milk lactoferrin

Bactericidal activity of human lactoferrin is distinct from its iron-withholding activity. Direct binding of lactoferrin to bacteria is though the high positive charges of lactoferrin molecule and can easily induce non-specific binding of lactoferrin to either bacteria or hosts. The molecular mechanisms of this bactericidal activity of lactoferrin, appears to be quite similar for both Gram-negative and positive bacteria, through damaging of bacterial membranes (Valenti and Antonini, 2005).

In Gram-negative bacteria, lactoferrin specifically binds to porins present on the outer membrane and induces the release of lipopolysaccharides and cause to increase bacterial susceptibility to osmotic shock, lysozyme and other antibacterial molecules. There are two ways for this case; lactoferrinis a poly cationic molecule with high surface positive charge in the N-lobe (Baker et al 2002). This positive region binds to the lipid A of lipopolysaccharides molecules on the outer membrane of bacterial species. Also, it is proved that lactoferrin can bind Ca^{2+}, releasing high amounts of lipopolysaccharides from Gram-negative bacteria without the need of direct contact with bacteria (Valenti and Antonini, 2005).

Lactoferrin also bind Ca2+through the carboxylate groups of the sialic acid residues on two glycan chains (Berlutti et al., 2004). Particularly the binding occure to the phosphate group within the lipid A, inducing a rigidification of the acyl chains of lipopolysaccharides (Orsi, 2004).

Antibacterial activity related to proteolysis

In addition to bactericidal activity, lactoferrin inhibits the growth of some bacteria such as *Shigella flexneri* and *E. coli* through degradation of proteins necessary for colonization of these bacteria (Orsi, 2004; Parker et al., 2015).

Degradation of *Haemophilus influenzae* IgA1 protease was observed by lactoferrin. Human lactoferrin degraded both the IgA1 protease and Hap adhesin by serine protease like activity of the N-lobe of lactoferrin. Lactoferrin inhibited enteropathogenic *E. coli* adherence, hemolysis and induction of actin polymerisation in Hep2 cells by degradation of proteins A, B and D (Esp ABD) of *E. coli* (Parker et al., 2015). Lactoferrin displays proteolytic activity against some bacterial virulence factors and decrease the pathogenicity of certain microorganisms (Valenti and Antonini, 2005).

Massucci et al. (2004) reported that proteolytic activity of bovine lactoferrin is similar to trypsin, and serine protease inhibitors prevent this catalytic activity. Interestingly, it appears less than 10% of the lactoferrin molecules possess proteolytic activity (Valenti and Antonini, 2005).

Lactoferrin enhances the uptake of pathogens

The presence of iron bound lactoferrin plays a vital role in enhancing the uptake of intracellular pathogenic bacteria such as *Mycoplasma*, *Mycobacterium*, *Chlamydia*, *Borrelia* which can be degraded by free radical ions or reactive oxygen species in RBCs and macrophages (Anand et al., 2015).

In addition, low expression of MDR was observed by iron saturated lactoferrin. Lower drug resistance of pathogens by increasing the sensitivity of resistant pathogens towards drugs and retaining the drug inside the cells works on eradication of these bacteria. Macrophages activated and show various metabolic activities and lead to inhibition of pathogens through phagocytosis. These cellular processes will be various by iron saturation levels of lactoferrin (Parker et al., 2015).

Influence of lactoferrinon adhesion on the cell surfaces and biofilm formation: The adhesion, colonizing and biofilm formation of microbes on host cell surfaces is a key step in the development and persistence of infections. Also, the high resistance of microbial biofilm to natural defense

mechanisms and antibiotics needs to find compounds that prevents bacterial adhesion. A large number of Gram-positive and negative bacteria possess specific adhesions that induce their adhesion to epithelial cells of host (Valenti and Antonini, 2005). Different effects of lactoferrin on bacterial aggregation and biofilm formation have been observed regarding to respiratory and oral infections (Valenti and Antonini, 2005).

Singh et al. (2002) reported that lactoferrin can be effective in the innate immunity by blocking the biofilm development by *Pseudomonas aeruginosa*. By iron binding ability, at concentrations lower than killing or preventing the growth of bacteria, lactoferrin induces twitching, as special form of surface motility, then the bacteria wander across the surface and don't form clusters or biofilms. The formation of biofilm is a very important step in the colonization of the host (Orsi2004).

Lactoferrin and respiratory infections: Cystic fibrosis (CF), is associated with alterations in the influx and efflux of chloride and sodium ions, results in very high concentrations of iron in sputum (Stites et al 1998; Valenti and Antonini, 2005). Increase in iron content and inducing of reactive oxygen species generation contribute to lung disorders, enhances the growth and colonization of *Pseudomonas aeruginosa* and *Burkholderia cepacia*, as two motile Gram-negative pathogens that are a major reason of morbidity and mortality of CF patients. Biofilm formation is major agent for virulence of both these bacteria. Peptides and proteins of natural non-immune defenses such as lactoferrin play vital role in combating such infections. Apo-lactoferrin, by chelating iron, inhibits *P. aeruginosa* adhesion and biofilm formation (Singh et al., 2004; Valenti and Antonini, 2005). Similarly to *P. aeruginosa*, free-living forms of *B. cepacia* also show a noticeable motility under iron-limiting conditions. It means, iron availability or the addition of iron-saturated bovine lactoferrin protective agents, and induces aggregation of *P. aeruginosa*and *B. cepacia* into biofilm in CF cases. The human lactoferrin concentration increases at higher concentrations than normal condition in infection and inflammation processes and also it is found in sputum of CF cases and chronic bronchitis patients (Thompson et al., 1990).

Lactoferrin and oral infections: In human saliva, the iron content ranges from 0.1 to 1.0 μM depending on meals, bleeding and oral pathologies. The physiological level of human lactoferrin in saliva varies from 5 to 20μg/ml and it will be reached to 60μg/ml during infections and inflammations. *Streptococcus mutans* in the human oral cavity is the principal etiological agent of dental caries, thus adhesion and aggregation capability of this bacteria cause to pathogenicity. Recently, apo-bovine lactoferrin in a saliva pool enhance *S. mutans* aggregates and biofilm formation, whereas iron-saturated bovine lactoferrin decreases aggregation and biofilm development (Berlutti et al., 2004). Saliva of caries-resistant patients through high aggregation efficiency and very low adhesion-promoting activity of these bacteria favors the clearance of bacteria.

Apo-human bovine lactoferrin induces aggregation of *Porphyromonas gingivalis* as an anaerobic Gram-negative bacterium, which is associated with periodontitis (Aguilera et al., 1998). However, in these patients, the high iron concentration and the presence of hemin and bovine lactoferrin degradation by bacterial enzymes (Alugupalli and Kalfas, 1996), could be responsible for the lack activity (Valenti and Antonini, 2005).

Conclusion

Milk lactoferrin especially camel milk lactoferrin can reduce bacterial growth, inhibit bacterial adhesion and biofilm formation; thus, it might be considered as antimicrobial therapeutic agent. Lactoferrin is able to bind iron, and hinder this nutrient for bacteria at the infection site and inhibit the growth of these microorganisms as well as the expression of their virulence factors. *In vitro* and *in vivo* studies have shown that lactoferrin prevent the attachment of certain bacteria to the host cells. Regarding the increasing resistance to antibiotics, it is necessary to explore novel antimicrobial drugs to bacterial diseases.

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