

# REVIEW ON THE PHARMACOLOGICAL ACTIVITIES OF LACTOFERRICIN AND LACTOFERRICIN ANALOGUES

Dimana Dimitrova<sup>1</sup>, Antonia Hristova<sup>1</sup>, Momchil Lambev<sup>1</sup>, Silvia Mihaylova<sup>1</sup>,  
Tamara Paipanova<sup>2</sup>, Stefka Valcheva-Kuzmanova<sup>3</sup>

<sup>1</sup>TRS Assistant Pharmacist, Medical College, Medical University of Varna

<sup>2</sup>Institute of Molecular Biology Acad. Roumen Tsanev,  
Bulgarian Academy of Sciences, Sofia

<sup>3</sup>Department of Pharmacology and Clinical Pharmacology and Therapeutics,  
Faculty of Medicine, Medical University of Varna

## ABSTRACT

**INTRODUCTION:** Antimicrobial peptides (AMPs) are a growing class of natural and synthetic compounds with a wide spectrum of targets including viruses, bacteria, fungi, and parasites.

**AIM:** The aim of the present review was to make an overview of the available literature on the pharmacological activities of these peptides, focusing on lactoferricin and lactoferricin analogues.

**MATERIALS AND METHODS:** To achieve this aim, information from databases, such as PubMed, Google Scholar and ResearchGate was searched and summarized.

**RESULTS:** Antimicrobial peptides (AMPs) are considered a promising alternative to the antibiotics used nowadays. They have drawn a special attention in the fight against infections caused by antibiotic-resistant bacterial strains. Lactoferricin is the most studied AMP derived from milk protein. Lactoferricin (Lfcin) is a fragment of the ferro-chelate complex of the bovine protein lactoferrin (Lf). The amphipathic and cationic properties of Lfcin account for its broad spectrum antimicrobial activity. Lfcin also manifests potential antiviral, immunomodulatory, antitumor and anti-inflammatory properties. Shorter analogues of Lfcins have been synthesized with a broad spectrum of activities and enhanced pharmacokinetic properties.

**CONCLUSION:** Lactoferricin and its analogues are a good demonstration that it is possible to design and obtain synthetic peptides with enhanced pharmacological activities.

**Keywords:** antimicrobial peptides, lactoferricin, lactoferricin analogues, pharmacological activities

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### Address for correspondence:

Stefka Valcheva-Kuzmanova  
Faculty of Medicine  
Medical University of Varna  
55, Marin Drinov Str.  
9002 Varna  
e-mail: stefkavk@yahoo.com

**Received:** April 3, 2019

**Accepted:** June 2, 2019

## INTRODUCTION

The increasing level of resistance to the antibiotics used nowadays is a serious threat to the public health. A lot of patients are hospitalized as a result of infections caused by resistant bacteria, such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, methicillin-resistant *S. aureus*, etc. (1). Antimicrobial peptides (AMPs) have received special attention as a possible alterna-

tive to fight against infections caused by antibiotic-resistant bacterial strains (2).

In the present paper, the pharmacological activities of synthetic peptides derived from Lactoferricin (Lfcin) are summarized on the basis of information from databases, such as PubMed, Google Scholar and ResearchGate.

#### **Antimicrobial Peptides (AMPs)**

AMPs can be isolated from both prokaryotes (bacteria) and eukaryotes (fungi, plants, insects and animals) (3). They are oligopeptides that contain a different number of amino acids (size from 2-10 kDa)

of the bovine protein lactoferrin (Lf). When Lf enters the gastrointestinal tract, it undergoes hydrolysis by the enzyme pepsin and turns into Lfcin (14). Lfcin can be found in humans (LfcinH), in cattle (LfcinB) and mice (LfcinM) (15).

Lfcin contains both hydrophobic and hydrophilic residues (16). It contains aromatic amino acids such as tryptophan (Trp) and phenylalanine (Phe), as well as some basic amino acids, such as arginine (Arg) and lysine (Lys). Lfcin contains two cysteine residues which make up an internal disulfide bridge and ensure the amphipathic properties of the peptide (Table 1) (15).

*Table 1. Amino acid sequences of bovine, mouse and human Lfcins. The highlighted residues (bold) present the availability of a disulfide bond between two cysteine residues in each peptide. Adapted from Vorland et al. (15)*

Lactoferricin (Lfcin)	Amino Acid Sequence
Bovine (LfcinB)	NH <sub>2</sub> -PHE-LYS- <b>CYS</b> -ARG-ARG-TRPGLN-TRP-ARG-MET-LYS-LYS-LEUGLY-ALA-PRO-SER-ILE-THR- <b>CYS</b> -VAL-ARG-ARG-ALA-PHE-COOH
Murine (LfcinM)	NH <sub>2</sub> -GLU-LYS- <b>CYS</b> -LEU-ARG-TRPGLN-ASN-GLU-MET-ARG-LYSVAL-GLY-GLY-PRO-PRO-LEU-SER- <b>CYS</b> -VAL-LYS-LYS-SER-SER-COOH
Human (LfcinH)	NH <sub>2</sub> -THR-LYS- <b>CYS</b> -PHE-GLN-TRPGLN-ARG-GLN-MET-ARG-LYSVAL-ARG-GLY-PRO-PRO-VAL-SER- <b>CYS</b> -ILE-LYS-ARG-ASP-SER-COOH

and demonstrate amphipathic properties. They usually have a positive charge (that ranges from +2 to +9) due to the abundance of the basic amino acids such as lysine (Lys) and arginine (Arg). This allows their selective binding to the negatively charged biological membranes of microorganisms (4,5). AMPs contain also other specific amino acids such as proline (Pro) (6), tryptophan (Trp) (7,8), glycine (Gly) (9,10), cysteine (Cys) (11) or histidine (His) (12).

Trp-rich AMPs (TrAMPs) are of a particular interest. The amino acid Trp has unique biochemical properties that account for the strong antimicrobial properties of TrAMPs. Representatives of TrAMPs are indolicidin, tritrypticin and Lfcin. Indolicidin possesses activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, *S. epidermidis*, *T. beigeli*, *C. albicans*, *S. cerevisiae*, HSV and *G. lamblia* (13). Tritrypticin is active against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. epidermidis*, *Streptococcus* group D, *P. mirabilis* and *A. fumigatus* (13).

#### **Lactoferricin Structure**

Lfcin is derived from milk protein. It is a 25-amino acid fragment of the ferro-chelate complex

#### **Lactoferricin Antimicrobial Activity**

Lfcin is a more potent antimicrobial agent in comparison with Lf (15) and its antimicrobial effect is due to its cationic and amphipathic properties (17-20). Lfcin binds to negatively charged components of bacterial membranes and disrupts the membrane integrity (21). The amino acid sequence in different types of Lfcin is related to the antimicrobial activity (Table 1).

A study of Vorland et al. (15) has shown that LfcinB has the lowest minimal inhibitory concentration (MIC) and is the only Lfcin that is active against *E. coli* and *S. aureus*. LfcinB demonstrates a stronger antimicrobial activity in comparison with LfcinH and LfcinM due to its greater hydrophobicity and the higher positive charge (15).

LfcinB-derived peptide (LfB17-41) has demonstrated activity against *E. coli*, *S. aureus*, *C. albicans*, *C. tropicalis*, *C. neoformans*, HCMV, HSV (13). The synthetic bovine Lfcin analogue (LfB17-31 or LfcinB17-31) acts against *E. coli*, *S. aureus*, *B. subtilis*, *S. cerevisiae*, *P. digitatum*, *P. italicum*, *P. expansum*, *Alternaria spp.*, *A. nidulans*, *B. cinerea*, *F. oxysporum*

and *M. grisea*. LfcinB20-25, another synthetic bovine Lfcin analogue, has a spectrum of activity including *E. coli*, *B. subtilis*, *S. cerevisiae*, *P. digitatum*, *P. italicum*, *P. expansum*, *Alternaria spp.*, *A. nidulans*, *B. cinerea*, *F. oxysporum* and *M. grisea*. The spectra of other bovine Lfcin analogues are respectively *E. coli*, *S. aureus*, *P. aeruginosa* for LfB17-27 and *E. coli*, *S. aureus* for LfB4-14.

### Lactoferricin Antiviral Activity

HSV infection is one of the most frequent viral infections in people. Its treatment started back in the 1970s with aciclovir. Research on the mechanism of action and the knowledge about the anti-HSV activity of Lf/Lfcin have been successfully applied in designing new, more specific anti-HSV agents (22). Lf competes for the heparan sulfate attachment sites in the host cells. As a result, viral entry inhibition and

Table 2. Mechanisms of the immunomodulatory and anti-inflammatory effects of bovine and human Lf and Lfcin (36)

Peptide and Origin	Immunomodulatory Effects
Bovine lactoferrin (LfB)	↓LPS-induced TNF- $\alpha$ in osteoblasts
	↓flagellin-induced IL-1 $\beta$ in osteoclasts
	↓TNF- $\alpha$ , IL-6, and GM-CSF in squamous cell carcinoma cell line
	IL-6 in LPS-treated THP-1 cells
	↑phagocytosis of BMDM, THP-1 and U937 cells, and neutrophils
	↓oxidative stress/apoptosis in U937 cells
	↑CD40 in RAW 264.7 cells
	↑IL-12 in peritoneal macrophages
	↓T-cell proliferation and secretion of IL-5
	↓TNF- $\alpha$ and IL-6 in blood of E.coli infected mice
	↓TNF- $\alpha$ and IL-6 in carrageenan-induced inflammation in rat feet
Bovine lactoferricin (LfcinB)	↓iNOS and IL-6 in nucleus pulposus cells of human intervertebral disc
	↓IL-1 $\beta$ , IL-6, iNOS, TLR-2 in chondrocytes
	↑IL-4 and IL-10 in chondrocytes
	↑IL-10 in articular cartilage
	↓IL-6 in LPS-stimulated THP-1 cells
	↑thymidine uptake by mitogen-activated splenocytes
Human lactoferrin (LfH)	↓ICAM, E-selectin and TNF- $\alpha$ in HUVECs
	↑IL-8, CXCL10, IL-10 in human DCs
	↓TNF- $\alpha$ in macrophages
	↓PGE2 secretion by breast milk macrophages
	↓IFN $\gamma$ in human DCs
	↓IL6 in LPS-stimulated THP-1 cells
	↓inflammation in dextran sulfate model of colitis in mice
	↓enteritis in <i>Shigella flexneri</i> treated rabbits
	↓epithelial cell proliferation
Human lactoferricin (LfcinH)	↓myeloperoxidase activity in primary human macrophages
	↑IL-10 in primary human macrophages
	↓TNF- $\alpha$ in LPS-stimulated human mononuclear cells
	↓TNF- $\alpha$ in macrophages stimulated with lipid A

suppression of the further HSV infection of the host cells occur (23-26). Lfcins display antiviral activity against other viruses, such as HPV (27) and HIV (28).

#### **Lactoferricin Antitumor Activity**

Lfcins display a highly selective cytotoxic effect against tumor cells. They target the membrane anionic phosphatidylserine on the cellular membrane of cancer cells and disrupt the membrane (29). Apart from this, the peptides which translocate to the cytosol, interact with the mitochondrial membrane due to its high content of negatively charged lipids. As a consequence, they disrupt the metabolism and may induce apoptosis. Apoptosis at a late stage is induced by LfcinsB by inhibition of autophagy (30). Bovine Lf and LfcinsB exert antitumor activities on human colorectal cancer cells (HT-29) (31). LfcinB induces apoptosis in human monocytic leukemia cell lines THP-1 (32). It can be attributed to the intracellular free radical production and activation of  $Ca^{2+}/Mg^{2+}$ -dependent endonucleases (33). *In vivo* tests have shown that bovine lactoferrin (LfB) and LfcinB suppress tumor growth and inhibit tumor metastases in mice (34). Apart from this, LfcinB has been reported to increase the sensitivity to anticancer drugs (35). This results from LfcinB-induced inhibition of glutathione S-transferase P1 isoform (GSTP1), which is overexpressed in some cancer cells and is responsible for their resistance to anticancer drugs.

#### **Lactoferricin Immunomodulatory and Anti-Inflammatory Activities**

Lf and Lfcin are peptides that are able to act as immunomodulators and anti-inflammatory agents as well (Table 2) (36).

The most in-depth research of the immunomodulatory effect of these peptides refers to their ability to suppress the proinflammatory response *in vivo* and *in vitro*. Lactoferrin modulates the migration, maturation and function of immune cells (37-39).

Research on human Lf (LfH) has shown the importance of this peptide as an anti-inflammatory agent. A study conducted by Haversen et al. has convincingly demonstrated the importance of LfH in reducing the IL-1 $\beta$ -provoked inflammatory response in mice that suffer from acute dextran sulphate-induced colitis (40).

LfcinB is also an effective anti-inflammatory and anti-catabolic agent. It can regulate the anti-inflammatory cytokines IL-6, IL-10, IL-11 and IL-4 (36,41). Investigations of LfcinH have also shown its ability to regulate cytokines (IL-10) and to reduce the production of TNF- $\alpha$  in macrophages induced by bacteria (42-44).

Some applications of Lfcins on the basis of their immunomodulatory, antimicrobial, antiviral and antitumor activities are summarized in Table 3 (15).

Table 3. Applications of Lfcins; adapted from Hao et al., 2018 (15)

Field	Application	Target	Object
Preclinical	Skin cancer	-	Mouse
	Keratitis	<i>Ps. aeruginosa</i> , <i>S. aureus</i>	Mouse
	Herpes simplex virus	HSV-2 line	Mouse
Veterinary	Atopic dermatitis	-	Dog
	Otitis externa	Bacteria and yeast	Dog
	Bovine mastitis	<i>Staphylococci</i> , <i>Streptococci</i> , <i>E. coli</i>	Cow
	Dietary supplementation	Antibiotic alternative, <i>E. coli</i>	Weaned piglet
Food	Spoilage	<i>Pseudomonas fluorescens</i>	Ground beef
	Spoilage	<i>Pseudomonas spp.</i>	Vegetable
	Spoilage	<i>Penicillium digitatum</i>	Mandarins
	Spoilage	Lactic acid bacteria, <i>Dekkera bruxellensis</i>	Wine
	Spoilage	<i>Pseudomonas spp.</i> , <i>E. coli</i>	Cheese

### Synthesis and Antimicrobial Activity of Lactoferricin Analogues

The design of peptides with potential pharmacological and therapeutic application is based on the synthesis of a series of structurally similar substances and tracing the relationship between structure and activity in standard pharmacological tests with the help of which their clinical efficacy can be predicted.

Peptides tend to draw a greater attention as potential drugs. Nevertheless, their application is limited because of some physicochemical characteristics. Such are the relatively high molecular mass and hydrophilicity, which lead to a limited penetration through biological membranes, as well as the degradation by peptidases and instability in the bloodstream. These properties determine a low bioavailability and short plasma half-lives. Among the chemical transformations of peptides, cyclization, the incorporating of D-amino acids or non-protein amino acids are of practical significance. Thus, the modified oligopeptides easily turn into derivatives with enhanced pharmacokinetic properties.

Recently, scientists have paid a great attention to new chemical structures which have biological activities. Analogues of the natural AMPs are being synthesized by incorporating both natural and non-natural amino acid sequences in their structure. Thus, shorter and more effective AMP derivatives are obtained. There is evidence already that AMP derivatives are able to effectively destroy pathogenic microorganisms (45). Cationic AMPs bind preferentially to the negatively charged phospholipid bilayer of bacterial membranes resulting in formation of transmembrane pores and ultimately bacterial cell death (19). The lack of specific receptors makes it difficult for bacteria to develop resistance to the peptide (46). Short peptides are supposed to demonstrate higher antimicrobial activities (45,47). The most important structural requirement for the manifestation of a high antimicrobial activity of such short peptides is the content of minimum 3 amino acid residues containing the amino acids Arg and Trp. Short peptides, which do not meet the above-mentioned requirement, demonstrate a much lower activity. Lfcins are one such example. The antimicrobial activity of Lfcin analogues as modular systems has been explored. Research is being conducted to find out which mo-

lecular characteristics are important for the antimicrobial activity. Data is available for observed antibacterial activity of the 25 amino acid fragment 17-41 of the ripe protein LfB (LfcinB). In 1996, it was shown that its undecapeptide segment 20-30 (LfB20-30) has a similar antimicrobial activity to that of the 25 amino acid fragment. The antibacterial activity of LfB20-30 is enhanced by modification that substitutes all lysine amino acid residues with arginine. The activity of the homologous undecapeptide segments 17-27 from LfcinB can be improved later with the incorporation of tryptophan amino acid residues (14). Even shorter Lfcin derivatives also manifest a good antibacterial activity. The active center of LfcinB is a hexapeptide corresponding to the 20-25 fragment. The amidation of the C-terminal of this peptide influences the potentiation of its activity in relation to *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria (48).

### CONCLUSION

The natural antimicrobial peptides can offer a good alternative to the conventional antibiotics, which have become highly ineffective against resistant bacterial strains. Lfcins have proven to be good antimicrobial agents (*in vitro*). Antiviral, immunomodulatory and antitumor activities of these peptides have also been established *in vitro* and *in vivo*. There is a possibility of potentiating the antimicrobial activity through synthesizing shorter analogues of Lfcins, which are good antimicrobial agents with a proven ability to avoid developing resistance to them.

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