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ANTIMICROBIAL PEPTIDES OF CAMEL MILK - A REVIEW

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ABSTRACT

Camel milk is rich in bioactive peptides, lactoferrin, zinc, monounsaturated fatty acids and polyunsaturated fatty acids, among other health substances. This article reviews the mechanism of action of antimicrobial peptides, as well as the types and different effects of antimicrobial peptides obtained by different treatment methods in camel milk. The antibacterial peptides in camel milk can not only act alone, but also act in the form of complexes, such as camel recombinant chiral lactoferrin+lactoferrin+His-tag, which has inhibitory effects on plant bacterial pathogens; In addition, there are many common antimicrobial peptides, such as peptidoglycan recognition protein (PGRP), lactoferrin, immunoglobulin, etc., which have inhibitory effects on various bacteria. Although, many antimicrobial peptides have been found to play important roles in food and medicine, there are still more unknown aspects that need to be explored.

Key words: Antibacterial mechanism, antibacterial peptides, camel milk

Many studies have demonstrated the production and properties of peptides from milk proteins. Several bioactive peptides have good health effects on digestive, immune, cardiovascular and nervous systems. Whey proteins represent about 30% of the total proteins in camel's milk (Zhao *et al,* 2015). Whey proteins such as IgGs, Lf, lactoperoxidase, lysozyme and other enzymes are potent antimicrobial components in camel's milk (El-Agamy *et al,* 1992). Antimicrobial peptides (AMPs) are active small molecular peptides that can be produced by all organisms. They are an indispensable part of the innate immune system and can limit the growth of other microorganisms (Magana *et al,* 2020). Natural antimicrobial peptides have broad-spectrum killing activity against a variety of bacteria, yeasts, fungi, viruses and parasites (Huy *et al,* 2020). Antimicrobial peptides not only resist pathogens, but also possess properties, such as anti-inflammation, immune regulation, neutralising endotoxin and so on (Hee-Kyoung *et al,* 2017). Antimicrobial peptides act on different bacterial structural targets through a variety of mechanisms, hence the drug resistance is relatively rare (Browne *et al,* 2020). Various mechanisms of bacterial resistance to antimicrobial peptides have emerged, including modification of cell surface components, degradation of antimicrobial peptides and efflux of antimicrobial peptides (Milad *et al,* 2019). This review will mainly summarise the antibacterial peptides and their effects in camel milk.

Antibacterial peptides obtained from different treatments of camel milk

Different treatments of camel milk can make it play different roles (Fig 1). For example, the protein in camel milk is fermented to produce new peptides that exert antibacterial effects. Various studies have shown that camel milk is rich in antimicrobial peptides, which have an inhibitory effect on a variety of bacteria. Some studies have also found that the new peptides cultured from fermented camel milk can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* subspecies (Hussein *et al,* 2021). Algboory *et al* (2017) fermented camel milk using a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii.* The peptide concentration in fermented camel milk (0.483mg/mL) was three times higher than that of the same fresh milk before fermentation (0.156mg/mL). The concentration of peptides in the water extract was 0.435mg/mL. Bacterial fermentation of Iraqi camel milk has the potential to increase water-soluble peptides and enhance biological activity.

New peptides with antibacterial activity can also be produced through recombinant chimerism.

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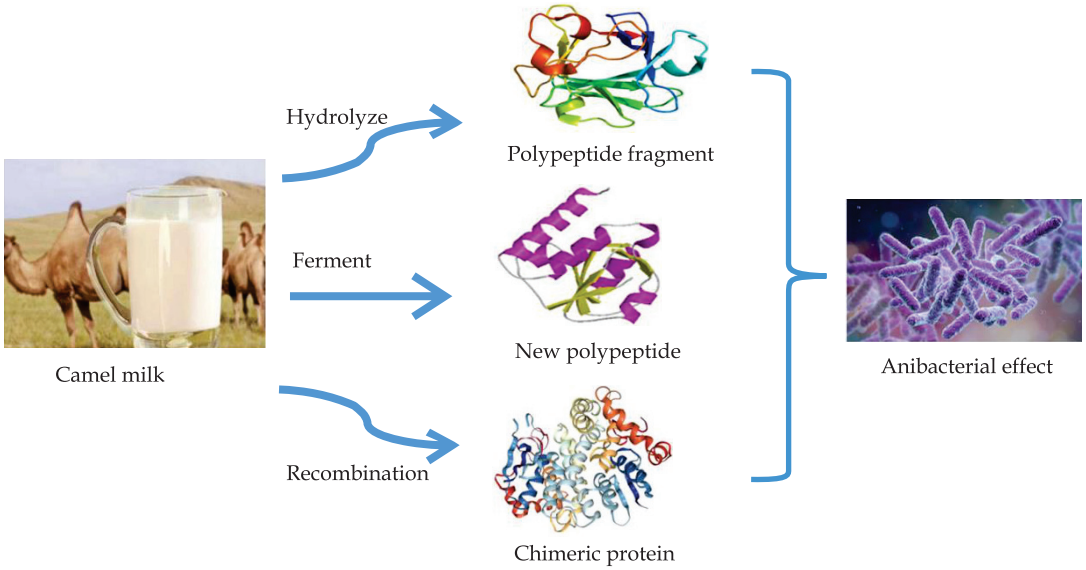

Fig 1. Camel milk obtains different antimicrobial peptides through different processing methods.

The LFA-LFC chimera has significant antibacterial properties against caries inducing bacteria and is not toxic to human gingival fibroblasts. Therefore, this peptide can serve as a safe alternative to other chemicals for the prevention and management of dental caries (Mohammadipour *et al,* 2021). Research has shown for the first time that the prepared camel recombinant chimeric lactoferrin+lactoferrin+His tag has good antibacterial activity against plant bacterial pathogens (Tanhaeian *et al,* 2018).

Different peptide fragments can also be produced through hydrolysis, which can exert antibacterial effects. *Trichosporon asahii ICVY021* were isolated from camel milk and found that it was able to inhibit *Kocuria rhizophila CIP* 53.45, through production of an extracellular heat-stable, proteinaceous antibacterial peptide,with partial amino acid sequences of PPFPK and CTHV(L/I)(K/Q) or TCHV(L/I)(K/Q), determined using LC/MS/MS. This peptide, named oranicin P16, was thought to impede the cell-division mechanism. Subsequent experiments also confirmed that the strain has the activity of anti-*K. rhizophila* (Soufian *et al,* 2020). It is to evaluate the potential antimicrobial effects of camel milk derived antimicrobial proteins and peptides against *Propionibacterium acnes* by micro broth dilution assay. The results showed that peptidoglycan recognition proteins PGRPs possess the strongest antimicrobial activity against *P. acnes* (Abu-qatouseh *et al,* 2019). Studies have shown that camel milk β-CN, the major protein of camel milk, can be hydrolysed with pepsin to increase its anti-bacterial activity. All fractions of hydrolysates exhibited some anti-bacterial activity and molecular mass fraction <1 kDa was the most active against both Gram- positive bacterial strains (Almi-Sebbane *et al,* 2018). The hydrolysates of camel cheese protein can be graded by ultrafiltration technology to obtain peptides with different molecular weight ranges, which have high antibacterial activity, such as anti *Listeria monocytogenes, Escherichia coli, Bacillus cereus, Staphylococcus argenteus* (Kumar *et al,* 2016). AMPs and proteins collected from camel milk have considerable antimicrobial and anti-inflammatory effects on *P. acnes* and would provide new trend toward applying these biological molecules in the management of acne vulgaris and other skin related infections (Shihab, 2019). Camel milk lactoferrin was used as the raw material and different AMPs were designed using bioinformatics methods. The most suitable AMP was screened according to the required standards. The designed peptide has antibacterial activity against *Pseudomonas aeruginosa, Baumannia* and *Staphylococcus aureus* (Khajeh *et al,* 2021). Lactoferrin (Lf) is an iron binding glycoprotein, which exists in different biological fluids and neutrophils of mammals. It has many functions, including protection from pathogen infections. The antibacterial activity of lactoferrin against *E. coli 0157: H7* was studied. The minimum inhibitory concentration (MIC) was determined by measuring the absorbance at 620nm. The minimum bactericidal concentrations (MBCs) were also measured. It was found that camel Lf was the most effective lactoferrin against *E. coli 0157: H7*, while camel and human lactoferrin had the lowest activity (Conesa *et al,* 2008).

Mechanism of action of antimicrobial peptides

Destruction of cell membrane function

The most basic mechanism of action of antimicrobial peptides is to destroy the bacterial plasma membrane structure, causing a large amount of water-soluble substances to seep out of the cell eventually leading to bacterial death (Dekker *et al*, 2001). The structural characteristics of antimicrobial peptide molecules are an important basis for ensuring the effectiveness of the aforementioned mechanisms. Bechinger (1997) proposed a model based on the interaction between antimicrobial peptide HNP2 and the hydrophobic and polar regions of biofilms, as well as the size of membrane pores formed. The model consists of 6 homologous dimers of defensin molecules forming a porous ring. Cheristensen *et al* (1988) studied the bactericidal mechanism of antimicrobial peptides using bilayer lipid membrane liposomes and believed that first, the positively charged nitroic acid of antimicrobial peptides and the negative charge formed by the bacterial cytoplasmic phospholipid molecules generate electrostatic attraction, causing the antimicrobial peptides to attach to the surface of the bacterial membrane. Then, the hydrophobic C-terminal is inserted into the hydrophobic region of the membrane and changes the conformation of the membrane. Multiple antimicrobial peptide molecules form ion channels on the membrane, leading to the loss of intracellular ions, especially the large escape of K+. Bacteria cannot maintain their normal osmotic pressure and die.

Inhibition of cellular respiration theory

Bobek *et al* (2003) used antibacterial peptide MUC7 extracted to act on common fungi, bacteria and cocci in clinical practice and found that MUC7 has a strong killing effect on both fungi and bacteria. In the ultrastructure, swelling, vacuolisation, ridge detachment and irregular arrangement of mitochondria were found, with unclear nuclear membrane boundaries and some nuclei ruptured and contents overflowed. This suggests that the mechanism of action of antimicrobial peptide MUC7 may be related to inhibiting tumour cell respiration. Fehlbaum *et al* (1996) also found that the mechanism of action of the antibacterial peptide tachy-plesin is related to the inhibition of mitochondrial related caspase7 and caspase6 proteins. Some researchers also believe that thanatin kills bacteria by inhibiting cellular respiration.

Inducing cell apoptosis

Mai *et al* (2001) injected the fusion antimicrobial peptide DP1 locally into solid tumours to study the effect of DP1 on the apoptosis of tumour cell line MCA20. They found that DP1 can quickly induce tumour cell apoptosis and reduce tumour volume. Chen *et al* (2001) treated prostate cancer cell line TSU with the antimicrobial peptide RGD tachyplesin and detected it using fluorescence immunoassay and Western blot hybridisation. The results showed that the expression of apoptosis related proteins caspase9, caspase8, caspase3 and Fas ligand increased, indicating that the antimicrobial peptide RGD tachyplesin can induce Fas related apoptosis. Therefore, it is inferred that inducing apoptosis

Table 1. Antibacterial peptides and their effects.

Antimicrobial peptides	Suppressed bacteria	Reference
FVVTPK, RGLVPL ELLPDMPLNQ APGPLVVPPVGPPPP PLPASGLL VMVSGVAGNPGA HPPGSGLL	*E. coli, S. aureus* subsp. aureus.	Hussein *et al* (2021)
lactoferrampin-lactoferricin [LFA-LFC]	*S. mutans, S. salivarius, S. sobrinus*	Mohammadipour *et al* (2021)
Camel recombinant chimeric lactoferricin + lactoferrampin +His-tag	plant bacterial, pathogens	Tanhaeian *et al* (2018)
PPFPK and CTHV(L/I)(K/Q) or TCHV(L/I)(K/Q)	*K. rhizophila*	Soufian *et al* (2020)
Lactoferrin, peptidoglycan recognition proteins (PGRPs) and immunoglobulins specific	*P. acnes* isolated	Abu-qatouseh *et al* (2019)
β-casein and β-CN hydrolysate	*E. coli, L. innocua, S. aureus*	Almi-Sebbane *et al* (2018)
Alcalase, α-Chymotrypsin	*L. monocytogenes, E. coli, B. cereus, S.aureus*	Kumar *et al* (2016)
peptidoglycan recognition proteins (PGRPs)	*P. acnes*	Shihab (2019)
Lactoferrin	*E. coli 0157:H7*	Conesa *et al* (2008)

may be one of the mechanisms of action of certain antimicrobial peptides.

Inhibition of cell wall formation

Harder *et al* (2001) found that antimicrobial peptides can inhibit the formation of bacterial cell walls, hinder bacterial growth and cause cell wall perforation, ultimately leading to bacterial death. Among the various mechanisms of action of antimicrobial peptides currently discovered, the membrane attack theory is considered to be the main mechanism of action of antimicrobial peptides. However, the same antimicrobial peptide may also exert its effects through multiple pathways. Antimicrobial cationic peptides can inhibit the formation of bacterial cell walls, preventing bacterial growth from maintaining normal cell morphology, but has no effect on already formed cell walls. Due to the different permeability of antimicrobial peptides to bacterial cell walls, the minimum lethal concentration of different antimicrobial peptides and the same antimicrobial peptide to different bacteria also varies greatly (Friedrich *et al,* 2000).

Effect on cancer cell cytoskeleton

At present, extensive research has been conducted on the killing effect of antimicrobial peptides on tumour cells and it has been found that the main effect of antimicrobial peptides on cancer cells cultured *in vitro* is to form pores on the membrane of cancer cells, cause leakage of contents and cause vacuolisation of mitochondria and detachment of cristae. The boundary of the nuclear membrane is unclear, with some cases of nuclear membrane damage, nuclear chromosome DNA breakage and inhibition of chromosome DNA synthesis, resulting in a certain degree of damage to the cytoskeleton. Antibacterial peptides can also stimulate the immune function and resist the invasion of cancer from the perspective of humoral immunity. Chen *et al* (2001) studied the anti-tumour mechanism of cecropin B, B1 and B3. Although, antimicrobial peptides have certain damage to the cytoskeleton of tumour cells and normal cells, the latter has a complete cytoskeleton system, fast repair and will not cause irreversible damage. Taghipour *et al* (2023) isolated camel milk protein components, casein and whey protein from antimicrobial peptides and hydrolysed them using pepsin, trypsin and these two enzymes. To screen peptides with antibacterial activity against breast cancer and pathogens. The peptides extracted from whey protein fractions using these two enzymes showed very good activity against MCF-7 breast cancer. The incomplete cytoskeleton of tumour cells, which cannot be repaired in a timely manner after the action of antimicrobial peptides, ultimately leads to death.

Application of antimicrobial peptides

Application of antimicrobial peptides in food

Antibacterial peptides have a strong killing effect on various Gram positive and negative bacteria in food (Tailor *et al,* 1997). Under acidic conditions, it has strong activity and can quickly inhibit the growth of microorganisms. It is suitable for most acidic foods. Proteases can quickly hydrolyse antimicrobial peptides consumed by humans and livestock and have no toxic side effects. Meanwhile, antimicrobial peptides have good thermal stability and solubility. In the process of food fermentation, antimicrobial peptides can effectively preserve certain bacterial communities (such as lactic acid bacteria) and can also cultivate or kill certain bacteria to prevent harmful bacteria. Antibacterial peptides can still maintain their unique physiological activity after hot processing.

Antibacterial peptides are also gradually being applied in the preservation of raw milk. In European countries, due to the distance between residential areas and pastures, milk needs to go through a long time during transportation in the hot summer. Insufficient refrigeration equipment can cause milk to spoil and cause great losses to producers. Adding a certain amount of antibacterial peptides to raw milk can effectively inhibit the spoilage bacteria produced in milk, extend the shelf life of milk without affecting its flavour.

Application of antimicrobial peptides in medicine

At present, more than 2500-3000 antimicrobial peptides have been isolated and identified, of which 261 have been confirmed to have anti-tumour activity (Wang, 2023). Antibacterial peptides or their precursor genes can be directly introduced into tumour cells, or these can be directly injected into the tumour to exert their effects. The high selectivity of the target audience of antibacterial peptides brings hope for the development of anticancer drugs, which may become a new type of peptide anti-tumour drugs that replace traditional surgery, radiotherapy and chemotherapy.

The currently used radiochemotherapy drugs can not only kill tumour cells, but also normal cells, with significant side effects. The development of the cytoskeleton system of tumour cells is incomplete and antimicrobial peptides can be inserted into the

cell plasma membrane, causing the microtubules of the cells to collapse, resulting in the dissolution of the bilayer and disruption of integrity. Antibacterial peptides can inhibit the growth of certain tumours with minimal toxic side effects and are harmless to normal cells, which brings great hope for the development of anti-tumour drugs. Antibacterial peptides can attack tumour cell membranes, forming pores on the cell membrane, causing a large amount of cell contents to seep out, ultimately causing tumour cells to fail to maintain normal osmotic pressure and die (Shai, 2002). Antibacterial peptides induce tumour cell death through the death receptor pathway (Chen *et al*, 2009) and mitochondrial pathway (Aarbiou *et al*, 2006). Antibacterial peptides can also damage the internal organelles of tumour cells, such as DNA breakage, mitochondrial damage and cytoskeleton breakage. The clinical application of antimicrobial peptides in anti-tumour has shown good prospects. At the same time, antimicrobial peptides can also enhance the body's immune system, resist tumour cell invasion and participate in cellular and humoral immunity. Due to its anti-tumour and antiviral properties, the development of skin antibiotics, antiviral drugs and anti-tumour drugs also has irresistible clinical application prospects, which will bring immeasurable significance to the development of disease treatment and medical health.

Conclusion

Antimicrobial peptides are an important component of biological innate immunity and have a wide range of killing effects on pathogens such as Gram negative bacteria, Gram positive bacteria, fungi and viruses, making them less susceptible to drug resistance. Camel milk antibacterial peptides are the most promising antimicrobial agents for addressing the challenge of multidrug-resistant bacteria and can be used alone or in combination with conventional antibiotics, antiviral drugs, or other antibacterial ingredients to achieve synergistic effects. Therefore, camel milk antimicrobial peptides are expected to become a new type of antibacterial growth promoter and immune modulator, applied in various industries, inducing or promoting the expression of antimicrobial peptides through nutritional regulation and increasing the concentration of local antimicrobial peptides in the mucosa, to achieve the goal of inhibiting pathogen invasion and improving the body's ability to resist infection, thereby reducing the use of antibiotics or dependence on antibiotics. Although, camel milk antimicrobial peptides have broad application prospects, research on camel milk antimicrobial peptides is still relatively limited, such as the toxicity, immunogenicity, pharmacodynamics and pharmacokinetics of camel milk antimicrobial peptides. The clinical trials of camel milk antimicrobial peptides are still limited to a certain aspect, with more basic research and less clinical and *in vivo* experiments. Therefore, in order to achieve the commercial application of camel milk antimicrobial peptides, a lot of basic work needs to be done.

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