World Journal of Microbiology and Biotechnology (2022) 38:39
https://doi.org/10.1007/s11274-022-03227-2

REVIEW

Indolicidin revisited: biological activity, potential applications and perspectives of an antimicrobial peptide not yet fully explored

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Received: 26 October 2021 / Accepted: 2 January 2022 / Published online: 12 January 2022

Abstract

The emergence of multidrug-resistant bacteria, viruses and tumors is a serious threat to public health. Among natural peptides, indolicidin, a 13-residue peptide belonging to the cathelicidin family, deserves special attention. Indolicidin has a broad spectrum of biological activity and is active against a wide range of targets, such as bacteria (Gram+ and Gram−), fungi and viruses. Here, we review the most important features of the biological activity, potential applications and perspectives of indolicidin and its analogs. Although not yet approved for commercialization, this peptide has great potential to be applied in different areas, including the medical, biomedical, food industry and other unexplored areas. To achieve this goal, a multidisciplinary team of researchers must work together to fine tune peptides that overall lead to novel analogs and formulations to combat existing and possibly future diseases.

Keywords Antimicrobial activity · Indolicidin · Indolicidin analogs

Antimicrobial peptides

Antimicrobial peptides (AMPs) are part of the innate immune system constituting the body's first line of defense for pathogen inactivation (Pasupuleti et al. 2012). Among the many ways to classify AMPs, one takes into account their biosynthetic mechanism. Thus, there are ribosomal and nonribosomal AMPs. The first are gene-encoded, ribosomally synthesized peptides that are widely distributed in nature. On the other hand, nonribosomal AMPs are produced by nonribosomal peptide synthetases not limited to the 20 proteinogenic amino acids (Liu et al. 2019). To date, more than 3000 AMPs (naturals and synthetics) have been included in different databases (Kang et al. 2019; Wang et al. 2016). The antimicrobial activity of AMPs is regulated by chemical and physical properties, including peptide charge, hydrophobicity, amphipathicity, and self-assembly equilibria (Shagaghi et al. 2018). AMPs have wide-spectrum activity being active against pathogenic microbes, including bacteria, fungi, parasites and viruses (Huan et al. 2020). In addition to their antimicrobial activity, AMPs are candidates as antitumor agents (Deslouches and Peter Di 2017). A very interesting feature of AMPs is the low tendency to resistance by the targeted cells (Datta and Roy 2021; Huan et al. 2020; Magana et al. 2020). This is mainly because most AMPs exploit fundamental features of bacterial cells, such as the plasma membrane (Maróti Gergely et al. 2011). However, several studies have shown diverse mechanisms of bacterial resistance to AMPs, such as alteration of membrane charge or fluidity, degradation, and removal by efflux pumps (Abdi et al. 2019; Joo et al. 2016).

Despite the promising features, AMPs have some issues to resolve, such as selectivity, stability and cost of production (Chen and Lu 2020). Fortunately, AMPs can be chemically modified so their biological activity and stability can be improved (Li et al. 2021). Between the thousands of AMPs reported, we highlighted indolicidin. In this review, we summarized the main features of indolicidin and its analogs to push the further application of these peptides in various fields. This is the first review entirely dedicated to indolicidin, a peptide not yet fully explored.

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Cathelicidins

The cathelicidin family, also named 'myeloid antimicrobial peptides', is a large group of AMPs found in a variety of mammalian species, such as humans and farm animals (bovine, porcine, caprine and chicken) (Kościuczuk et al. 2012; Tomasinsig et al. 2010). Cathelicidins are stored in the secretory granules of neutrophils and macrophages and are released upon leukocyte activation. These peptides are small, cationic, amphipathic and show a broad spectrum of antimicrobial activity. They are processed from precursors (prepropeptides) that are synthesized in myeloid bone marrow cells (Kastin 2013). The mature peptides vary markedly in sequence and length, ranging from 12 to 100 amino acid residues. Cathelicidin encoding genes contain four exons. The first exon encodes the conserved signal peptide (29–30 amino acids), whereas the second and third exons encode the major part of the cathelin-like domain (94–114 amino acids). Finally, the fourth exon encodes the last few amino acid residues of the cathelin domain and is hypervariable, encoding the heterogenous mature peptide, with the variable antimicrobial domain consisting of 12–100 amino acids, with very limited sequence similarity between species (Fig. 1).

C-terminal mature peptides could be activated by proteolytic cleavage and exert antimicrobial and immunomodulatory activities after being released from the N-terminal cathelin portion (Zanetti 2004). The exact mechanism of processing is still not understood, as the unprocessed propeptide can be found extracellularly as a variety of processed forms (Kastin 2013). Based on amino acid sequences, mature cathelicidin peptides after the protease cleaving steps are quite diverse and can be broadly categorized into cyclic dodecapeptides with one disulfide bond, porcine protegrins with two disulfide bonds, peptides with an α-helical structure, peptides containing a high number of tryptophan, proline and arginine residues, and short tandemly arranged peptides.

The mechanism of action of most cathelicidins, such as that of other AMPs, involves interactions with cell membranes. This action is based on the interaction of cationic residues and the negatively charged lipid membranes of microorganisms (Ramanathan et al. 2002). Cathelicidins can also act on other targets, such as protein synthesis and DNA replication. On the other hand, cathelicidin's immunomodulatory activity is mediated through its interaction with cells that are part of the innate immune system, such as monocytes, dendritic cells, T cells and epithelial cells (Bowdish et al. 2006). Other known biological functions of cathelicidins include wound repair, angiogenesis and chemotaxis (Kościuczuk et al. 2012).

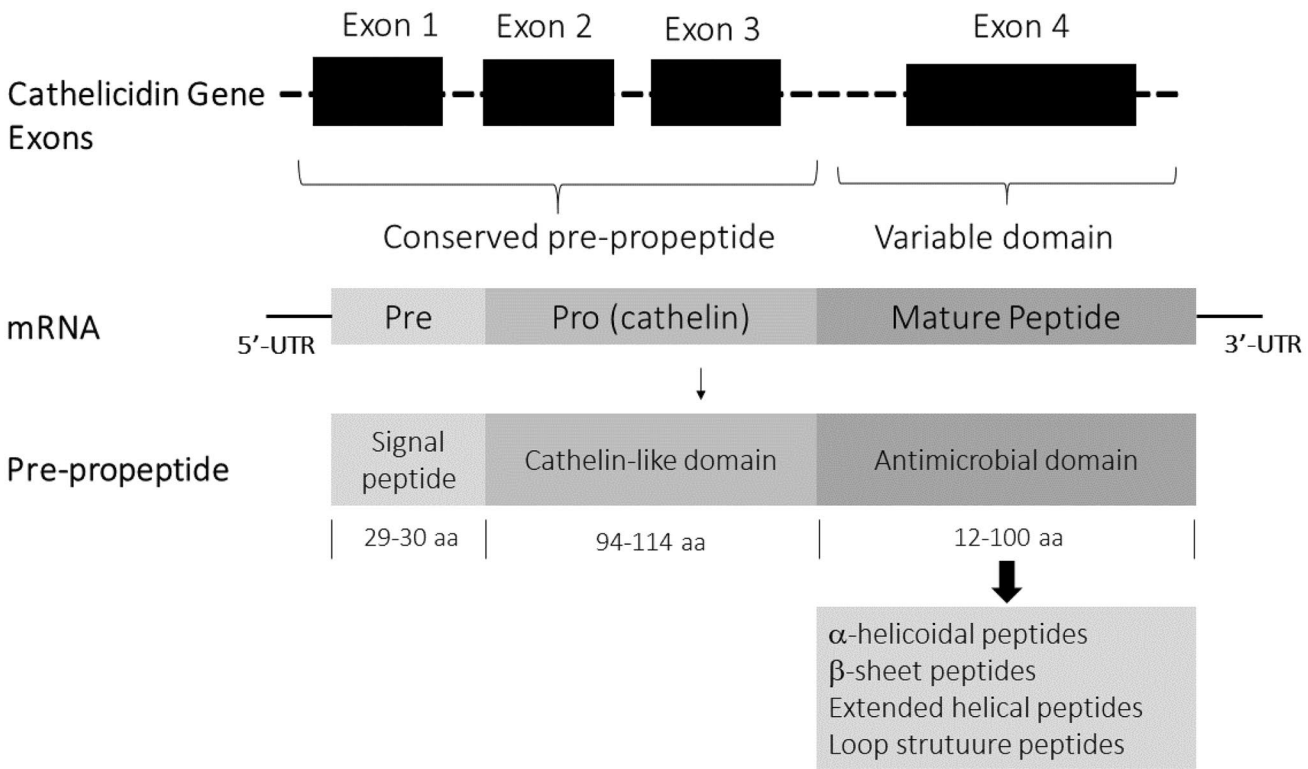

Fig. 1 Cathelicidin gene structure and processing. Adapted from Kastin (2013) and Wang et al. (2020)

The story of the cathelicidin family began when the first cathelicidin, cecropin, was isolated in 1980 from *Hyalophora cecropia* (Hultmark et al. 1980) and continue with magainin, isolated in 1987 by Zasloff from the skin of the *Xenopus leavis* frog. Later, Scocchi et al. in 1997, characterized the bovine cathelicidin gene family on the basis of molecular cloning and sequence analysis of cDNAs. According to Tomasinig et al. (2010), the different types of cathelicidins expressed in bovines, including indolicidin, may contribute to the integrated response to infection.

Indolicidin

Indolicidin, which was discovered by Selsted et al. (1992), is a natural peptide belonging to the cathelicidin family isolated from bovine neutrophils. The peptide has 13 amino acid residues, almost half of which are hydrophobic, including 5 residues of tryptophan. Tryptophan residues are responsible for insertion and partitioning in biological membranes, leading to the hemolytic activity of the peptide (Subbalakshmi et al. 1996). The sequence and physicochemical properties of indolicidin are presented in Table 1.

The structure of indolicidin (in several environments) has been studied using different biophysical and theoretical techniques. Structural characterization using circular dichroism and ^{1}H NMR spectroscopy supported the absence of any secondary structure for indolicidin (Podorieszach and Huttunen-Hennelly 2010). Furthermore, molecular dynamics simulations indicated that the peptide adopts a boat-shaped conformation in negatively charged lipid bilayers (Hsu and Yip 2007). The differentiated amino acid composition of indolicidin determines multiple spatial conformations of the molecule. This "structural plasticity" appears to be important in the biological activity of the peptide (Hsu et al. 2005). This plasticity also ensures the recognition of a broad spectrum of microorganisms (Nagpal et al. 2009).

Table 1 Sequence and physicochemical properties of indolicidin

Sequence	ILPWKWPWWPWRR-NH_2
Structure	Multiple spatial conformations
Residue number	13
Molecular weight	1906.3
Net charge*	+4
Isoelectric point	14
Solubility	1 mg/ml (water)
+Synthesis	Easy

*Net charge at pH 7: Lys (K), Arg (R), and C-terminal amidation (NH_2) were assigned with + 1 charge. +Synthesis: based on our experience using manual SPPS (for more details see Supplementary Data)

Indolicidin's targets

In terms of biological activity, indolicidin is also a unique peptide since it has a wide range of biological targets, including Gram-positive (Gram+) and Gram-negative (Gram−) bacteria (Vergis et al. 2020). Antibacterial activity is not confined to planktonic organisms, and there are also several studies showing antibiofilm activity (de Alteriis et al. 2018; Mataraci and Dosler 2012). Additionally, several researchers found antifungal and antiparasitic activities for indolicidin (de Alteriis et al. 2018; Haines et al. 2003). Furthermore, certain viruses are inactivated by indolicidin (Krajewski et al. 2004; Ron-Doitch et al. 2016). Indolicidin has also been evaluated in combination with conventional antibiotics with the aim of evaluating potential synergism (Ghaffar et al. 2015; Mataraci and Dosler 2012). Finally, Bacalum (2016) reported activity against neuroblastoma SH-SY-5Y.

Planktonic microorganisms

Most of the studies on AMP activity, including indolicidin, are conducted on planktonic microorganisms. Table 2 shows microorganisms, minimal inhibitory concentration (MIC) values and references of studies conducted with indolicidin. The wide spectrum of activity translates into a wide spectrum of applications, including human and animal health, the food industry and others.

Biofilms

Pathogens are able to adapt to different conditions and develop self-defense mechanisms, such as living in biofilms (Galdiero et al. 2016). Attachment to a living (mucosal and soft tissues) or nonliving surface (medical devices such as catheters) is common for several microorganisms. The clinical importance of biofilms is related to their structure, which grants more tolerance to antibiotics and disinfectants than the planktonic form. Antibiofilm activity is commonly addressed in two ways: the inhibition of biofilm formation and the destruction of the preformed biofilm. Interestingly, indolicidin has shown both activities. Alteriis et al. (2018), demonstrated that indolicidin causes 20–50% inhibition of biofilm formation and 10–60% destruction of preformed biofilms, both produced by fungi (*Candida albicans* and *Candida tropicalis*). Furthermore, indolicidin can also destroy and inhibit bacterial biofilms formed by resistant *Escherichia coli* and *Staphylococcus aureus* (Mataraci and Dosler 2012; Vergis et al. 2020).

There is a high demand for developing novel molecules to treat biofilm-associated microbial infection (Pervin and

Table 2 Nonexhaustive list of indolicidin activity against several microorganisms

Microorganism	Activity (μg/ml)	References
Staphylococcus aureus (*S. aureus*)	MIC 2–30	Dwivedi et al. (2019) Falla et al. (1996), Friedrich et al. (2000), Joshi et al. (2010), Kim et al. (2009), Nan et al. (2009b, 2009a), Park et al. (2009), Pompilio et al. (2011), Ryge et al. (2004), Subbalakshmi et al. (2000, 1996), Vasilchenko et al. (2017) and Yang et al. (2002)
Methicillin-resistant Staphylococcus aureus (MRSA)	MIC 8–15	Friedrich et al. (2000), Mataraci and Dosler (2012), Park et al. (2009), Shin et al. (2010) and Yan and Hancock (2001)
Staphylococcus haemolyticus	MIC 2	Friedrich et al. (2000)
Staphylococcus epidermidis	MIC 4–20	Dwivedi et al. (2019), Falla et al. (1996), Friedrich et al. (2000), Nan et al. (2009b, 2009a), Park et al. (2009) and Yang et al. (2002)
Enterococcus faecalis	MIC 30–60	Friedrich et al. (2000) and Yan and Hancock (2001)
Listeria monocytogenes	MIC 3–60	Friedrich et al. (2000), Shin et al. (2010) and Vasilchenko et al. (2017)
Streptococcus pyogenes	MIC 4	Friedrich et al. (2000)
Mycobacterium tuberculosis	MIC 30–120	Portell-Buj et al. (2019)
Escherichia coli (*E. coli*)	MIC 5–30	Bera et al. (2015), Dwivedi et al. (2019), Falla et al. (1996), Galdiero et al. (2016), Joshi et al. (2010), Kim et al. (2009), Nan et al. (2009b, 2009a), Park et al. (2009), Ryge et al. (2004), Shin et al. (2010), Subbalakshmi et al. (2000, 1996), Vasilchenko et al. (2017), Vergis et al. (2020), Yan and Hancock (2001) and Yang et al. (2002)
Pseudomonas aeruginosa (*P. aeruginosa*)	MIC 8–100	Bera et al. (2015), Dwivedi et al. (2019), Falla et al. (1996), Galdiero et al. (2016), Joshi et al. (2010), Nan et al. (2009a, 2009b), Park et al. (2009), Pompilio et al. (2011), Shin et al. (2010), Vasilchenko et al. (2017), Yan and Hancock (2001) and Yang et al. (2002)
Salmonella spp.	MIC 8–100	Bera et al. (2015), Falla et al. (1996), Nan et al. (2009b), Park et al. (2009), Vasilchenko et al. (2017) and Yang et al. (2002)
Klebsiella spp.	MIC 4–15	Bera et al. (2015), Dwivedi et al. (2019), Galdiero et al. (2016) and Shin et al. (2010)
Bacillus subtilis	MIC 2–30	Dwivedi et al. (2019), Joshi et al. (2010), Kim et al. (2009), Nan et al. (2009b, 2009a), Park et al. (2009), Shin et al. (2010) and Yang et al. (2002)
Micrococcus luteus	MIC 4–25	Kim et al. (2009) and Shin et al. (2010)
Bacillus cereus	MIC 10	Vasilchenko et al. (2017)
Candida albicans (*C. albicans*)	MIC 15–200	Benincasa et al. (2006), de Alteriis et al. (2018), Giacometti et al. (1999) and Yang et al. (2002)
Candida utilis	MIC 20–25	Subbalakshmi et al. (1996)
Saccharomyces cerevisiae	MIC 15–30	Benincasa et al. (2006) and Subbalakshmi et al. (1996)
Candida krusei	MIC 10–25	Benincasa et al. (2006) and Giacometti et al. (1999)
Candida tropicalis (*C. tropicalis*)	MIC 30–150	Benincasa et al. (2006), de Alteriis et al. (2018) and Giacometti et al. (1999)
Candida glabrata	MIC 60–90	Benincasa et al. (2006) and Giacometti et al. (1999)
Cryptococcus neoformans	MIC 4–45	Benincasa et al. (2006) and Giacometti et al. (1999)
Pneumocystis jirovecii	IC_{50} 40	Cirioni et al. (1998)
Human immunodeficiency virus (HIV)	IC_{50} 70–110	Edward Robinson et al. (1998) and Krajewski et al. (2004)
Herpes simplex virus (HSV)	IC_{50} 10–50	Albiol Matanic and Castilla (2004), Ron-Doitch et al. (2016) and Yasin et al. (2000)
Cryptosporidium parvum	Inhibition of 40% in 50%	Cirioni et al. (1998) and Giacometti et al. (1999)
Trypanosoma brucei	Inhibition of 100% in BSF (120–250) and PCF (250–500)	Haines et al. (2003)

MIC minimal inhibitory concentration, IC_{50} half maximal inhibitory concentration) values are presented as the lowest-highest values from references, *BSF* bloodstream forms, *PCF* procyclic culture forms

Hassan 2021). Evaluation of antibiofilm activity is underrated and constitutes a research field still less explored for indolicidin and its analogs.

Lipopolysaccharide interaction

Lipopolysaccharide (LPS) is considered an endotoxin and the major outer surface membrane component of almost all Gram-bacteria and acts as a powerful stimulant of the immune system (Cohen 2002). LPS activates inflammatory cells such as monocytes and macrophages, causing them to release cytokines (e.g., TNF-a, IL-1b and IL-6) and nitric oxide that trigger an inflammatory cascade (Nan et al. 2009a; Park et al. 2009). Interestingly, indolicidin has been shown to bind and neutralize LPS molecules with similar affinity than polymyxin B, defensins and cecropin–melittin hybrids (Falla et al. 1996; Nan et al. 2009a). It was demonstrated that the conformational plasticity of indolicidin contributes to binding. Hydrophobic interactions involving fatty acid chains of LPS and aromatic residues of indolicidin, plus salt bridges between arginine side chains of the peptide and phosphate groups of the LPS inner core, explain indolicidin–LPS binding (Nagpal et al. 2009). Because of the ability to bind and neutralize LPS, indolicidin is considered to be a potential anti-sepsis agent (Dwivedi et al. 2019). Additionally, this property is linked with the antibiofilm activity of indolicidin because LPS is one of the major virulence factors mostly involved in the production and adhesion of biofilms produced by Gram-bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Similar to LPS, lipoteichoic acid (LTA) present in Gram+ bacteria has been proven to be involved in the release of cytokines and biofilm production (Schröder et al. 2003). To the best of our knowledge, there are no studies evaluating the potential interaction of indolicidin with LTA, constituting an opportunity for research.

Mechanism of action

The elucidation of the mechanism of action of an AMP is important because it allows the understanding of biophysical processes that lead to microorganism death. This information is useful because it provides the basis for developing peptide analogs with improved features. Different strategies have been used to understand the mechanism of action of AMPs, such as spectroscopic techniques, nuclear magnetic resonance, isothermal titration calorimetry, molecular dynamics and others (Galdiero et al. 2013). In the case of indolicidin, studies related to its mechanism of action began with the work of Falla et al. (1996). The authors demonstrated that the peptide was capable of killing Gram-bacteria by crossing the outer membrane and causing disruption of the cytoplasmic membrane. Later, in 1998, Subbalakshmi and Sitaram demonstrated that the mechanism of action of indolicidin does not involve cell lysis. According to the authors, indolicidin permeabilizes both the outer and cytoplasmic membranes of *E. coli* but does not lead to lysis. Instead, once in the cytoplasm, indolicidin inhibits protein and DNA synthesis (Subbalakshmi et al. 2000). A few years later, Hsu et al. (2005), confirmed the DNA-binding ability of indolicidin using different experimental procedures, such as gel retardation, fluorescence measurement, and surface plasmon resonance. Similar to LPS, indolicidin–DNA interactions are mediated by the plasticity of the peptide. Finally, Rokitskaya et al. (2011), in a very interesting study conducted on uncharged lipid vesicles, concluded that indolicidin can operate as an organic anion carrier. The authors related this activity to the hemolytic action of indolicidin. The multiple mechanisms of action of indolicidin are shown in Fig. 2.

Even with the data described above, the mechanism of action of indolicidin is not yet fully understood. The complete elucidation of the mechanism of action of any AMP depends on several factors, such as the model used to mimic the real target and the peptide concentration (Gregory et al. 2009; Wang et al. 2011). In addition, the multiple targets of indolicidin contribute to the difficulty of completely elucidating the molecular basis of indolicidin's mechanism of action. Furthermore, the antiviral activity of indolicidin remains unclear.

Combination with conventional antibiotics

Several research groups have evaluated the potential of AMPs to work as adjuvants to conventional antibiotics (Li et al. 2020). The mechanisms underlying the synergistic behavior depend on the specific kind of antibiotic evaluated. For instance, the synergism between membrane-active AMPs combined with conventional antibiotics is related to the membrane-disruptive ability of the first to assist the second to reach their molecular targets more rapidly and efficiently (Shang et al. 2019). Studies conducted by Mataraci and Dosler have demonstrated that indolicidin in combination with daptomycin, linezolid, ciprofloxacin and azithromycin presents an additive interaction against MRSA stains. Additionally, indolicidin combined with teicoplanin showed a synergistic interaction (Dosler and Mataraci 2013; Mataraci and Dosler 2012). Furthermore, Ghaffar et al. (2015) showed that the combination of indolicidin with levofloxacin improves the antimicrobial activity against Gram+ and Gram− bacteria by decreasing the MIC values in relation to both separately. The valuation of indolicidin synergism with conventional antibiotics also constitutes an opportunity that researchers can explore further.

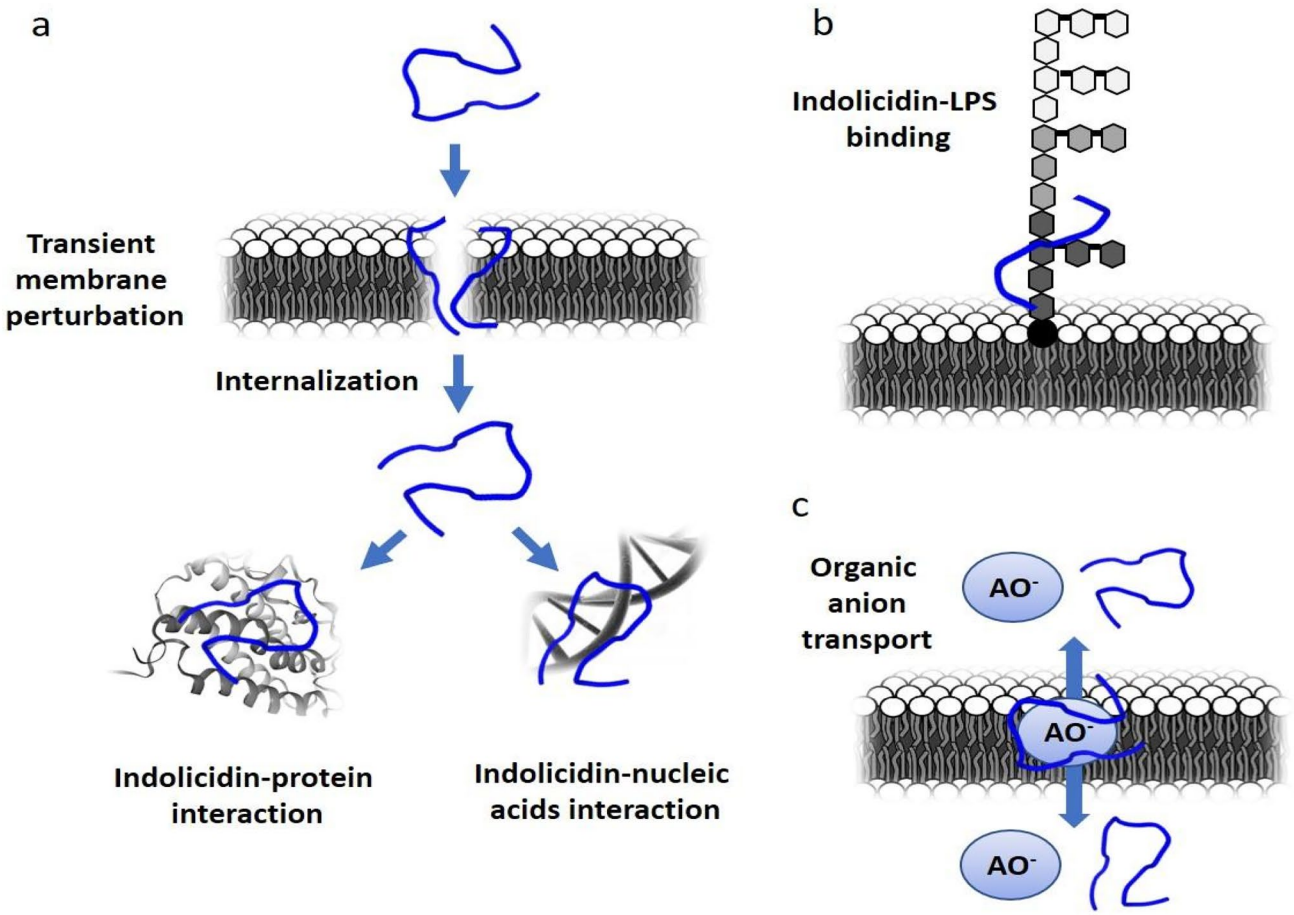

Fig. 2 Representation of indolicidin's mechanism of action after transient membrane perturbation, intracellular potential targets are proteins and nucleic acids (**a**), indolicidin–LPS interaction related to anti-sepsis and anti-biofilm activity (**b**) and the indolicidin anion carrier mechanism is thought to explain hemolytic activity (**c**)

Table 3 Indolicidin-inspired peptides with improved antimicrobial activity

Indolicidin analog	Antimicrobial activity*	Hemolytic activity*	References
ILKKWPWWPWRRK-O-CH_3	Increase (1–8×)	Decrease (3×)	Falla and Hancock (1997)
I**C**LKKWPWWPWRR**C**KNH_2	Increase (1–2×)	Decrease (8×)	Rozek et al. (2003)
ILRWPWWPWRRK-amide (Omiganan)	Increase (1–3×)	ND	Sader et al. (2004)
I ψ[CH_2NH] LPWKWPW ψ[CH_2NH] WPWRR-NH_2	Equal	Decrease (4×)	Kim et al. (2009)
(WWPWRR)2K-NH_2	Increase (1–2×)	Decrease (100×)	Shin (2013)
Ranalexin–indolicidin hybrids	Increase (1–8×)	Decrease (2–4×)	Jindal et al. (2015)
H-Ile-Leu-Pro-(2-Me)Phe-Lys-(2-Me)Phe-Pro-(2-Me)Phe-(2-Me)Phe-Pro-(2-Me)Phe-Arg-Arg-NH_2 and HN_2-$(CH_2)_{10}$-Ile-Leu-Pro-D-Phe-Lys-D-Phe-Pro-D-Phe-D-Phe-Pro-DPhe-Arg-Arg-NH_2	Increase	Decrease	Smirnova et al. (2020)

The results are presented as the fold change values related to indolicidin biological activity

*(1–nX) means that for some microorganisms, the activity was the same (1), while for others, it had an increase of "n" times

ND not determined

Indolicidin's inspired peptides

Natural peptides are usually models for the design of AMP analogs to obtain new molecules with improved activity. Its biological features plus its small size encouraged several research groups in the design of indolicidin analogs. In addition to an increase in antimicrobial activity, the researchers' goal is to decrease the hemolytic activity of indolicidin. A comparison between indolicidin and selected analogs is presented in Table 3.

The race for analogs started immediately after the discovery of indolicidin. It began with the study of Falla et al. (1997). Researchers designed an indolicidin analog with an increased number of positively charged residues by the use of lysine and arginine. The molecule, called CP-11, has higher activity against Gram+ and Gram− bacteria and yeast than its parent peptide. Furthermore, the peptide has less toxicity than indolicidin. The increased charge and amphipathicity could explain the improved activity.

A few years later, inspired by CP-11, Rozek et al. (2003), designed cycloCP-11 (ICLKKWPWWPWRRCK), a cyclic disulfide-bonded peptide. The major goal was to increase protease resistance. CycloCP-11 proved to be 4.5 times more stable than CP-11 against trypsin, mainly because cycloCP-11 had a more compact packing of the lysine and tryptophan side chains. Cyclic and linear CP-11 were similar in terms of microbiological and hemolytic activity, both better than indolicidin. In 2004, Sader et al. published a study in which they designed and evaluated omiganan (ILRWPWWPWRRK) against 1437 clinical bacterial and 214 clinical yeast isolates. Omiganan was very effective against all Gram+ and Gram− bacteria tested, including the antibiotic-resistant strains. Additionally, omiganan demonstrated excellent antifungal activity against all *Candida* species. Furthermore, a study showed that omiganan has a rapid time kill for both bacterial and yeast strains. To date, omiganan has been tested in a total of sixteen clinical studies in the United States and Europe. Almost all of them have been completed. Omiganan was studied to evaluate its efficacy as a topical gel formulation to treat Rosacea for the treatment of catheter colonization and the prevention of bloodstream infections, genital warts and acne vulgaris (for more details, see the corresponding clinical trial identifiers: NCT00231153, NCT03091426, NCT02849262, NCT02571998 and NCT02576847).

Later, in 2009, Kim et al. conducted a study in which amide bonds at various positions in indolicidin were replaced with reduced amide bonds $\psi[CH_2NH]$. The pseudopeptide containing two reduced amide bonds was less hemolytic without a decrease in its antimicrobial activity, probably related to the decreased hydrophobicity and enhanced conformational flexibility compared to indolicidin. Furthermore, the analog gained stability, an important feature for AMPs..

In 2013, Shin et al. tested a lys-linked dimeric analog of indolicidin (Di-Ind-6). This peptide displayed a two- to fourfold increase in antimicrobial activity against Gram+ and Gram− bacteria. The authors did not report information about the mechanism of action of the dimeric peptide. The factors that lead a monomeric peptide to become a more active dimeric molecule are not well established (Lorenzon et al. 2019). Additionally, Di-Ind-6 proves to be more hemolytic than Ind-6 but less hemolytic than native indolicidin. Furthermore, Di-Ind-6 inhibited nitric oxide production similar to the parent peptide.

Later, in a very complete set of experiments, Jindal et al. (2015) studied indolicidin–ranalexin hybrid peptides. Between 13 promising hybrids, RN7-IN10, RN7-IN9, RN7-IN8, and RN7-IN6 showed the strongest antibacterial activity against *Streptococcus pneumoniae*. Interestingly, all the hybrid peptides showed very low toxicity against human red blood cells. Later, in 2017, the authors conducted experiments that suggested that the cell wall/membrane or the inhibition of DNA synthesis are the most likely targets for hybrid peptides against *S. pneumoniae* (Jindal et al. 2017).

In a recent study, Smirnova et al. (2020) designed two analogs of indolicidin that have increased antimicrobial activity and decreased hemolytic activity. One of them is the peptide H-Ile-Leu-Pro-(2-Me)Phe-Lys-(2-Me)Phe-Pro-(2-Me)Phe-(2-Me)Phe-Pro-(2-Me)Phe-Arg-Arg-NH_2, an analog containing methylated phenylalanine residues instead of tryptophan. The other analog, HN2-(CH_2)10-Ile-Leu-Pro-D-Phe-Lys-D-Phe-Pro-D-Phe-D-Phe-Pro-DPhe-Arg-Arg-NH_2, has D-enantiomer phenylalanine residues (also instead of tryptophan) and ten carbon fatty acids at the N-terminus. The modifications in these analogs made them more hydrophobic than the parent peptide indolicidin, suggesting that their mechanism of action is based on the disruption of cell membranes, which is mostly dependent on hydrophobicity.

As described, several strategies can be applied to design more efficient indolicidin analogs. However, other promising strategies of peptide modifications and novel formulations have not yet been explored. It is evident that many new analogs can still be designed using indolicidin as a template. Li et al. (2021) have reported a complete set of peptide chemical modifications to enhance antimicrobial activity, including lipidation, glycosylation and multimerization.

Perspectives

The studies of indolicidin began in 1992 as described above and continue to the present day (Hamed and Seleem 2021; Selsted et al. 1992). More than a hundred scientific papers

were published between 1992 and 2020. Furthermore, a considerable number of patents can also be found, evidencing the interest of industry. However, similar to other AMPs, indolicidin and its analogs must overcome some limitations, such as instability, selectivity and production cost. Biological instability is considered the main issue for most peptides, specifically when systemic application is considered. However, it is important to note that many peptides, including indolicidin, act relatively quickly on their targets, and the natural elimination of the peptide by the body can be considered an advantage, avoiding the adverse effects of tissue accumulation (common for many conventional antibiotics). Indolicidin, like other AMPs, has hemolytic activity. Thus, topical administration of indolicidin is more likely than systemic application. In this regard, indolicidin analogs could be designed to increase selectivity toward desired targets. Alternatively, nanosystems for the delivery of indolicidin could be a promising alternative. A good peptide delivery platform could increase the peptide half-life time and targeting capability, improving its therapeutic applicability and reducing side effects.

Solid-phase peptide synthesis (SPPS) is still the standard method for producing synthetic peptides. However, several authors consider the high cost of production one of the reasons that keeps the industry away from interest in synthetic peptides. However, indolicidin, unlike other peptides (long and difficult sequences to synthesize), can be synthesized in high yields by SPPS. Conventional protocols for the use of 9-fluorenylmethoxycarbonyl amino-protecting group and standard coupling methods (Jaradat 2018) can be used to obtain indolicidin in high yield. Recently, we obtained indolicidin by manual SPPS with high yield and purity (Supplementary Data, not published). Furthermore, recombinant synthesis could be used to reduce the cost, obtain peptides on a large scale and reduce the use of hazardous materials.

In summary, there are still many strategies that researchers can take advantage of to improve the performance of indolicidin and, finally, be approved for commercialization. Despite several potential applications described in the literature, there are still some others that could be better addressed by the utilization of indolicidin and its analogs, such as antitumoral activity and gene delivery. There are few studies evaluating the potential antitumoral activity of the peptide. Cationic AMPs, such as indolicidin, are able to interact with negatively charged phosphatidylserine (PS) moieties that are selectively exposed on the outer surface of cancer cell plasma membranes (Tornesello et al. 2020). The negative charge of the cancer cell membrane is a characteristic also shared by bacterial cells. This fact constitutes the basis of the hypothesis of a close similarity between the selectivity and activity of AMPs and anticancer peptides (Gaspar et al. 2013). To date, only Bacalum (2016) has tested the antitumor activity of indolicidin. The author suggested that the peptide can inhibit cell growth of neuroblastoma SH-SY-5Y cells by apoptosis. The molecular basis of the mechanisms of action of indolicidin is still under debate but apparently is related to membrane penetration. Taking this into account, indolicidin and its analogs have the potential to act as carriers to deliver small molecules (Tsai et al. 2015). In this context, Hu et al. (2018) grafted indolicidin with polyethylenimine to investigate the potential of these conjugates as a gene delivery system. Researchers have shown that this peptide-conjugated carrier is an effective gene vehicle.

The potential use of indolicidin is not limited to biomedical applications but could also be used in different areas, such as the food industry (food conservation), veterinary (animal infection control, sperm/oocyte conservation, etc.) and other unexplored areas.

Conclusions

Indolicidin is a unique peptide with the potential to fight against the emergence of multidrug-resistant microorganisms, being active against bacteria, fungi, parasites and certain viruses. The multiple targets of indolicidin reinforce its potential therapeutic applications and could contribute to low resistance induction. Added to its broad spectrum of activity, ease of synthesis and good solubility position, indolicidin (and certain analogs) is an excellent candidate in the growing market for bioactive peptides. Like almost all AMPs, indolicidin has to overcome its limitations, mainly instability and hemolytic activity. In this regard, several strategies for peptide tuning (such as punctual modifications and multimerization) have not yet been fully explored. On the other hand, new formulations including nanosystem delivery could be developed. Indolicidin and its analogs could be used in different areas, including medical (infectious disease control), biomedical (such as medical device contamination control), food industry (food conservation) and other unexplored areas. To achieve this goal, a multidisciplinary team of researchers must work together to fully understand the chemistry and biological properties of indolicidin.

Supplementary Information The online version of this article (https://doi.org/10.1007/s11274-022-03227-2) contains supplementary material, which is available to authorized users.

Acknowledgements The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support (427961/2018-1) and John Oluwafemi Teibo for English revision.

Author contributions ENL devised the review and the main conceptual ideas and wrote the manuscript with help from JBA and GSS.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Not required.

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