Cecropins Activity Against Bacterial Pathogens

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Abstract: Today, resistant bacteria are challenges in the treatment of bacterial infections. Cecropins are compounds that kill bacteria by affecting the membrane of microorganisms, such as *Acinetobacter*, *Escherichia coli*, and *Staphylococcus*. This study aimed to evaluate the antibacterial activity of cecropins against bacterial pathogens quantitatively. In this study, articles reporting antimicrobial activity of cecropins were searched in PubMed, Web of Science, and Scopus databases using the Google Scholar search engine. Then, the results of the current study were evaluated quantitatively. In this study, we found 29 studies reporting cecropins antimicrobial activity against major bacterial pathogens. Also, there were 25 studies on cecropin antimicrobial activity against gram-negative pathogens, and it was cleared that cecropin B antibacterial activity on *Pseudomonas aeruginosa* was lesser than others (minimum inhibitory concentration, 0.4 μg/ML), and we showed that *Staphylococcus aureus* growth can be inhibited by Cecropin AD more than others (minimum inhibitory concentration, 0.2 μg/Ml). Because cecropin peptides have no adverse effect on the human cells, and also, it has been demonstrated that cecropins have acceptable functions against pathogenic bacteria, we showed that they are potential candidates for research and construction of novel antibiotics.

Key Words: cecropins, peptides, antibacterial agents, resistance

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At present, antimicrobial resistance is rapidly expanding throughout the world, so that the effectiveness of routine antibiotics has been questioned. Spreading antibiotic resistance and emergence of new resistant strains have created an urgent need to find a suitable alternative to antibiotics. In recent years, antimicrobial peptides (AMPs), which are components of the immune system, have been identified as effective antimicrobial agents for killing antibiotic-resistant bacteria. Antimicrobial peptides kill bacteria through 2 mechanisms. Antimicrobial peptide activity induces permeation in membranes of some bacteria, but in others, these compounds readily enter the cell membrane and interact with intracellular components, including nucleic acids.[1] An antimicrobial peptide is a cationic peptide and can affect gram-positive and gram-negative bacteria and mycobacterium growth.[2–4] Cecropins are antibacterial peptides that were extracted originally from insects[5]; they consist of 31 to 37 amino acids, and can affect gram-negative and gram-positive bacteria and mycobacteria. Bactericidin, lepidoptin, and sarcotoxin are other names of these structurally related proteins.[6] These peptides are secretory proteins and can be activated after deletion of the signal peptides, affecting a broad spectrum of gram-negative and gram-positive bacteria.[7] Tuberculosis is another infectious disease with which many people, such as pregnant women[8] throughout the world have been affected, and, in some cases, there is resistant to routine treatment mycobacterium. So, we searched antimycobacterial peptide killing *Mycobacterium* spp, and we found a few studies reporting antimycobacterial activity against *Mycobacterium* spp.[9,10] Antiendotoxin activity is another property of cecropins that has been reported in a study.[11] The most important types of cecropins are cecropin A, cecropin B, and cecropin P1. So far, a few studies have been performed on the antibacterial effect of cecropins on bacterial pathogens.[12,13] Cecropin A includes the KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK amino acid sequence, in which the secondary structure consists of 2 α helices.[2] Cecropin A, like other cecropins can affect the microbial cytoplasmic membrane, and this property has been demonstrated. In one study, antibacterial activity of cecropins was determined against multidrug-resistant nosocomial isolates of *Acinetobacter baumannii* compared with melittin and cecropin A-melittin peptide CA.[14] Of course, it is necessary to mention all kinds of cecropins can damage microbial membrane by 1 mechanism, and they can affect both gram-positive and gram-negative pathogens. Cecropin B amino acid have been sequenced (KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL) and structured by 2 α helices.[2] Cecropin B was extracted from the giant silkmoth, *Hyalophora cecropia*, by researchers and its antibacterial activity have been determined. This peptide is belonging to innate immune system and is effective protein in clearance and promotion of wound repair.[15]

Furthermore, the antibacterial activity of this peptide has been evaluated against both gram-negative and gram-positive bacteria.[16,17] The antibacterial activity of cecropin B also is related to the interaction with bacterial LPS so that disruption of LPS causes killing of bacteria. Cecropin B also inhibit production of cytokines nitric oxide and release of tumor necrosis factor-α in the host. So, these reports confirm that cecropin B have a potential in antibacterial and anti-inflammatory activities.

Finally, cecropin P1 (CP1) is a 3.3-kDa, 31-amino acid, that amino acid sequence contains SWLSKTAKKLENSAKKRISEGI AIAIQGGPR. This antibacterial peptide has been isolated from Ascaris, an intestinal nematode of pigs.[1] In one study, antibacterial mechanism of cecropin P1 have been shown, in which cecropin P1 can obstruct bacterial membranes instantaneously, and also, it was cleared that CP1 antimicrobial activity is time- and dose-dependent and but CP1 permeability begin at low concentrations.[18] In another study, there is evidences in which CP1 can be active against important infectious bacteria, such as *Pseudomonas aeruginosa*[19] and antibacterial resistance for cecropin P1 could not be considered because CP1 action on bacterial membrane is performed physically. Here we can mention a property of CP1 and that is lytic action without hemolysis.[20]

Although the antimicrobial profile of many antibiotics[21–23] and other antimicrobial agents[24] in exposure to bacterial pathogens have been determined so far, no comprehensive efforts have been made for AMPs till now. Therefore, the aim of our study was reporting in vitro quantitative activity of cecropin A, cecropin B, and cecropin P1 against multidrug-resistant bacteria.

MATERIAL AND METHODS

In the present study, we searched articles in Chemical Abstract, EBSCO, Scopus, PubMed, ISC, Web of Science, and

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TABLE 1. Quantitative Antimicrobial Activity of Cecropins Against Gram-negative Bacteria

			Antibacterial Activity			
Gram Negative Bacteria	**Strain**	**Cecropin**	**(MIC μg/mL)**	**(MBC μg/mL)**	**Other (μg/mL)**	**References**
P. aeruginosa	PA14	Cecropin A	32	ND	ND	25
	PA103		64	ND	ND	
	PA 2326		64	ND	ND	
	PA 1026		32	ND	ND	
	PAO1		64	ND	ND	
	PA 1016		>64	ND	ND	
	OT97		ND	ND	2.6	26
		Cecropin B	ND	ND	1.5	
	PAO1	CEME	2	ND	ND	27
		CE	>64	ND	ND	28
	ATCC 27853	BP100	33.5	ND	ND	29
		Cecropin P1	>256	ND	ND	30
		Cecropin B	64	ND	ND	
		CE	>64	ND	ND	28
	Clinical	Cecropin P1	4–64	8–128	ND	31
	ATCC27853	Cecropin B	0.40	ND	ND	32
		Cecropin P1	0.8–1.60	ND	ND	
Pseudomonas	*Pseudomonas fluorescent*	Cecropin 1	1.1	ND	ND	33
		Cecropin 2	1.9	ND	ND	
		Cecropin 3	1.3	ND	ND	
K. pneumoniae	ATCC77326	Cecropin A	4	ND	ND	25
	ATCC 700603 (MDR)	BP100	17	ND	ND	29
	Clinical strain	Cecropin P1	0.25–2	0.50–4	ND	31
Escherichia coli	OP50	Cecropin A	4	ND	ND	34
	WT		0.5	ND	ND	
	WT GFP		0.5	ND	ND	
	Δ waaP GFP		0.5	ND	ND	
	Δ waaC GFP		0.5	ND	ND	
	Δ waa F		0.25	ND	ND	
	Δ waa I		0.25	ND	ND	
	Δ waa Y		0.25	ND	ND	
	MG1655		0.9	ND	ND	
	D31		64–78	ND	ND	21
	Clinical		2.5	ND	ND	35,36
		Cecropin B	2.5	ND	ND	
		Cecropin p1	0.1–4	ND	ND	
	DH5 α	DAN1	4.9	ND	ND	37
		DAN2	2.1	ND	ND	
	Standard	Cecropin A	0.8–5	ND	ND	38
	D21		ND	ND	0.4	26
		Cecropin B	ND	ND	0.6	
	SC9251	Cecropin B	2	ND	ND	39
	UB1005	CEME	1	ND	ND	27
	D31	Cecropin A	1	ND	ND	40
	G	Cecropin A, B	0.1	ND	ND	41
	K12	Cecropin-like protein	0.15	ND	ND	42
	Clinical	Cecropin A	3	20	ND	43
	DH5α	Cecropin 1	0.1	ND	ND	33
		Cecropin 2	0.5	ND	ND	
		Cecropin 3	0.2	ND	ND	
	ATCC 25922	Cecropin A	64	ND	ND	28
	Coli UB 100	Cecropin A	>64	ND	ND	

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TABLE 1. *(Continued)*

Gram Negative Bacteria	Strain	Cecropin	Antibacterial Activity (MIC μg/mL)	(MBC μg/mL)	Other (μg/mL)	References
	ER2566	Cecropin B2	6.8	ND	ND	44
	BL21		7.2	ND	ND	
	Rosetta		7.2	ND	ND	
	JM109		6.6	ND	ND	
	DH1		6.7	ND	ND	
	K12D31	Cecropin AD	1.8	ND	ND	45
	K88		2	ND	ND	
	K99		2	ND	ND	
	UB100	CEME	2	ND	ND	46
	ATCC25922	Cecropin P1	16–32	ND	ND	30
		Cecropin B	16–32	ND	ND	
		BP100	17	ND	ND	29
	Clinical strain	Cecropin P1	0.25–1	0.25–2	ND	31
		Cecropin B	16	ND	ND	47
	ATCC25922	Cecropin B	0.4–1.60	ND	ND	32
		Cecropin P1	0.8–1.60	ND	ND	
	HB10	Cecropin B	0.10	ND	ND	
		Cecropin P1	0.8–1.60	ND	ND	
Acinetobacter baumannii	ATCC 17978	Cecropin A	2	ND	ND	25
	ATCC19606		4	8	ND	48
	GIM1.650		4	8	ND	
	Ac157	Cecropin A-Melittin	2	ND	ND	49
	BCRC 15884	Cecropin B2	6.8	ND	ND	44
	E1359		6.9	ND	ND	
	ATCC BAA-1605 (MDR)	Cecropin-α-melittin (BP100)	17–67	ND	ND	29
	Colistin susceptible		8.5–18	ND	ND	
Acinetobacter spp	Clinical strain	Cecropin P1	0.50–2	1–4	ND	31
		Cecropin B	16	ND	ND	47
Shigella sonnei	JS1 1746	Cecropin-like protein	0.08	ND	ND	42
Shigella spp.	clinical	Cecropin P1	1–32	2–32	ND	
Proteus vulgaris	OX19	Cecropin-like protein	0.3	ND	ND	
S. typhimurium	ATCC 14028	CE	>64	ND	ND	28
	ATCC 7731		>64	ND	ND	
	Clinical strain	Cecropin AD	8	ND	ND	45
Salmonella typhi	Clinical strain	Cecropin P1	0.50–8	1–8	ND	
S. enteritidis	Clinical strain	cecropin AD	16	ND	ND	
S. entericaser. TyphimuriumL	T2	Cecropin P1	>128	ND	ND	30
		Cecropin B	32	ND	ND	
Brucella spp	Clinical strain	Cecropin P1	0.25–2	0.50–2	ND	31
Francisella spp	*F. novicida*	Cecropin A1	ND	ND	20.1	22
		Cecropin B	ND	ND	4.64	

ND, not determined.

MedLib databases. Our search strategy was included: ("Cecropin "(All Fields)) AND (peptide"(All Fields)) AND resistance (All Fields) AND Bacteria (All Fields) AND Antimicrobial (All Fields) and keywords selected from Medical Subject Headings thesaurus. Articles were searched online and without time limitation, and inclusion criteria for articles in this study were based on report of quantitative evaluation of minimum inhibitory concentration minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), cecropins, and bacterial pathogens. In contrast, exclusion criteria included inability to access the full text and reports containing unrelated results. Data analysis in this review study was performed based on the reports, and data analysis methods or tools such as SPSS were not used.

RESULTS

In this study we found 71 articles which had reported the antimicrobial profile of cecropins and derivatives quantitatively. After examination of related articles, it was revealed that 29 studies had quantitative reports of antimicrobial activity of cecropins against bacterial pathogens based on MIC, MBC, and lethal dose. There were 25 studies on cecropin antimicrobial activity against

TABLE 2. Quantitative Antimicrobial Activity of Cecropins Against Gram-positive Bacteria

Gram-positive Bacteria	Strains	Cecropin	(MIC μg/mL)	(MBC μg/mL)	Other (μg/mL)	References
Staphylococcus aureus	RN4220	CEME	4		[47]	
	MW2	Cecropin A2	>64	ND	ND	25
	MRSA ATCC43300	CAMA	8	ND	640	50
	Standard	Cecropin 1	2.1	ND	ND	33
		Cecropin 2	5	ND	ND	
		Cecropin 3	2.5	ND	ND	
	ATCC29213	DAN1	4.9	ND	ND	37
		DAN2	2.1	ND	ND	
		Cecropin A	>64	ND	ND	28
		Cecropin P1	>256	ND	ND	30
		Cecropin B	>256	ND	ND	
	MRSA	Cecropin P1	>128	>128	ND	31
	MS	Cecropin P1	32–.128	32–.128	ND	
	MR	Cecropin P1	8–128	16–128	ND	
	IVDC C56005	Cecropin AD	0.2	ND	ND	45
	25923	CEME	8	ND	ND	46
	SAP0017 (MRSA)		4	ND	ND	
	Clinical isolate		8	ND	ND	
	Clinical isolate		4	ND	ND	
	ATCC 33591 (MRSA)	Cecropin-α-melittin hybridBP100	134.5	ND	ND	29
	ATCC9144	Cecropin P1	>100	ND	ND	32
	clinically resistant	Cecropin B	25	ND	ND	
			32	ND	ND	47
Streptococcus faecalis	IVDC C55614	Cecropin AD	24	ND	ND	45
Enterococcus faecium	E007	Cecropin A2	>64	ND	ND	25
	ATCC 700221 (VRE)	Cecropin-α–melittin hybridBP100	67	ND	ND	29
Enterococcus faecalis	ATCC 29212	Cecropin A	>64	ND	ND	28
		CEME	32	ND	ND	46
		Cecropin P1	>256	ND	ND	30
		Cecropin B	>256	ND	ND	
	Clinical isolate	Cecropin P1	>128	>128	ND	31
S. epidermidis	ATCC12228	Cecropin A	>64	ND	ND	28
	Clinical isolate	CEME	8	ND	ND	46
S. pyogenes	ATCC 19615		8	ND	ND	
Listeria. monocytogenes	NCTC 7973		4	ND	ND	
	N22–2	Cecropin P1	>256	ND	ND	30
		Cecropin B	>256	ND	ND	
Streptococcus pneumoniae	Clinical isolate	Cecropin P1	8–128	8–128	ND	31

MS, methicillin-susceptible; MR, methicillin-resistant; MRSA, methicillin-resistant *S. aureus*; VRE, vancomycin-resistant *Enterococcus*.

gram-negative pathogens and it was cleared that cecropin B antibacterial activity on *P. aeruginosa* was lesser than others (MIC, 0.4 μg/mL) (Table 1), 11 studies against gram-positive pathogens and we found *Staphylococcus aureus* growth can be inhibited by Cecropin AD more than others (MIC, 0.2 μg/mL) (Table 2), and 3 studies against mycobacterial pathogens (Table 3).

DISCUSSION

Cecropins are known antimicrobial cationic peptides that can inhibit the growth and activity of bacterial pathogens. These cationic peptides were extracted originally from insects,[21] but later, it was revealed that cecropins are a part of mammalian host innate immunity and probably can affect pathogenicity of microbial infection.[22–24] These results are according to our study findings as our results showed cecropins are antibacterial peptides that can inhibit important strains of pathogenic bacteria. Mechanisms of this antibacterial AMP have been determined first in Christensen et al[51] study, and it seems a strong positive charge by cecropins can form specific channels in the bacterial membrane by negative charge. It is necessary to mention that accordance to[52] Li et al study, cecropins are not toxigenic for human cell membranes due to lower negative charge in mammalian cell membranes. And in the Schweizer study,[53] it has been revealed that cecropin B have selective toxicity. So, if appropriate

TABLE 3. Quantitative Antimicrobial Activity of Cecropins Against *M. tuberculosis*

Strains	Cecropin	(MIC μg/mL)	(MBC μg/mL)	Other (μg/mL)	References
Mtb H37Rv	Cecropin P1	50,000	ND	ND	9
Mtb H37Ra	Cecropin B	600	ND	ND	10
CI74		1200	ND	ND	
CI85		1200	ND	ND	
CI114		600	ND	ND	
ClI121		1200	ND	ND	
CI		>120	ND	ND	4
	Cecropin A	>120	ND	ND	

CI, clinical isolate.

concentration of cecropins is adjacent to the bacterial membrane, the membrane will be altered due to difference in charge, and channels will be formed that disrupt the osmotic balance of the bacteria.[54] So high concentration of some cecropin such as cecropin B reported in the present study against important bacteria, such as *Mycobacterium tuberculosis,* could be considered as an antibiotic candidate (MIC>120).

Also, in the present study we reported the antimicrobial effects of cecropin based peptides on human pathogenic gram-negative bacteria, that is according to Moore et al study.[12] We evaluated cecropins activity on gram negative bacteria and it was found that cecropins can inhibit gram negative bacteria growth that have been mentioned in Table 1. For example, it was cleared that cecropin B antibacterial activity on *P. aeruginosa* was 0.4 μg/mL. After exanimation of evaluated articles, we found that some cecropins had antimicrobial activity also against gram-positive bacteria, according to Moore et al Wang et al[12,55] studies, that have been mentioned in Table 2. In the present study result showed that gram positive pathogens such as *Staphylococcus aureus* growth can be inhibited by 0.2 μg/mL of Cecropin AD. Furthermore, our data showed that cecropins B, P1 can inhibit Mycobacterium genus growth in high concentration of cecropins. This result is according to Siemion et al,[56] Linde et al,[9] and Portell-Buj et al[4] examination results. Our study showed that some cecropin peptides such as cecropin AD (MIC = 0.2 μg/Ml) and cecropin P1 (MIC = 0.25–2 μg/Ml) could be considered a safe antibacterial agent for human infection treatments in a safe concentration. We found that gram-negative bacteria, such as Escherichia coli, P. aeruginosa, and *Acinetobacter baumannii*, can be killed by cecropins. Also, it was revealed that cecropins had antibacterial property against Enterococci, Staphylococcus aureus, and Streptococcus pyogenes. Effective antibacterial activity of cecropins depends on several factors, such as the kind of bacterial strain and standard dosage of cecropins. Some strains, such as multidrug-resistant *A. baumannii* and *K. pneumoniae*, were inhibited by the high dosage of cecropins, but in the low dosage only susceptible strains had been inhibited. But, in resistant gram-positive bacteria, such as methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus*, cecropin EME, and BP100 had opposite properties. Inhibition of susceptible strains by lower dosage of cecropins is in accordance with another study performed on AMP.[57] Tuberculosis is another infectious disease by which many people throughout the world have been affected, and in some cases, there is resistant to routine treatment mycobacterium. Therefore, we searched antimycobacterial peptide killing *Mycobacterium* spp., and we found a few studies reporting antimycobacterial activity against *Mycobacterium* spp.[9,10]

After examination of more searched articles, we found that there were no further similar studies conducted on quantitative evaluation of cecropin antibacterial profile. However, we found a few articles reporting in vitro antimicrobial activity of cecropins on bacterial pathogens[58–61] and on antimicrobial activity of AMPs against viruses and fungi.[60]

Studies on cationic peptides have increased recently. Because these proteins have no adverse effects on human cells and have also shown an acceptable function against pathogenic bacteria, they are known as potential antibiotic candidates. Today, a number of these proteins, such as cecropin based peptides D2A21 and D4E1 have been entered into clinical trials assessment.[62,63] Therefore, it is proposed that other kinds of cecropin peptides be evaluated in vitro and in vivo and in animal models.

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