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SAMA peptide, a rationally designed antimicrobial peptide

Mohammad Alsaggar* iD, Alaa Al-Tarawneh, Yara Al Tall, Mohammad Al-Hazabreh

Department of Pharmaceutical Technology, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan.

ABSTRACT

Antimicrobial peptides (AMP) are gaining increasing attention as a potential alternative to existing antibiotics to tackle bacterial resistance. Herein, we aimed to design and examine a novel antimicrobial peptide with superior antimicrobial activity and safety profiles. The design relied on a hybridization approach of selected parts of two naturally existing peptides; the brevinin-1E and bombolitin IV peptides were used to design a novel peptide named SAMA, which was characterized *in silico* prior to experimental approaches. The antimicrobial and antibiofilm activities of SAMA were examined *in vitro* on representative control and multi-drug-resistant bacterial strains, alone or combined with five traditional antibiotics. The SAMA peptide displays strong and broad-spectrum antimicrobial effects against selected strains at concentrations of 5 to 15 µM. Moreover, 30–40 µM of the peptide was sufficient to eradicate the biofilm-forming Gram-positive strains. Combining the SAMA peptide with traditional antibiotics displayed a synergistic or additive mode of action, which significantly reduced their needed MIC values. In addition, SAMA displayed potential anticancer properties against the adenocarcinoma cell line. Importantly, therapeutic concentrations of SAMA displayed minimal hemolytic activity. Therefore, this study demonstrated that AMP indeed offer favorable substitutes to current antibiotics for the management of infectious diseases. The SAMA peptide is a broad-spectrum AMP and a promising candidate for antimicrobial and anticancer drug development.

INTRODUCTION

Bacteria are becoming increasingly resistant to conventional antibiotics, resulting in the therapeutic failure of many antibiotics, even newly introduced agents (Cornaglia, 2009; Tan, 2008). The emerging crisis of antibiotic resistance throughout the world is largely attributed to poor patient compliance to treatment guidelines, overprescription, and misuse of anti-infective agents in medical institutions and antibiotic abuse in agriculture (Palumbi, 2001). Additionally, several pharmaceutical companies abandoned the market of antibiotics (Alanis, 2005). Several approaches have been proposed to tackle the development of antibiotic resistance, such as the introduction of combined therapies targeting multiple bacterial targets, the evolvement of bacteriophage therapies, microbiota transplantation, and targeting of key resistance players, such as inhibition of drug-modifying enzymes and drug efflux pumps (Kaur, 2016). However, these

approaches resulted in limited success, typically due to cost, safety issues, and/or a narrow spectrum of efficacy. Therefore, increasing demand for new types of antimicrobial therapies to manage infectious diseases and bacterial resistance is reshaping drug discovery research and development.

Antimicrobial peptides (AMPs) are broadly expressed in nature, and they have been found in most living species as a part of the innate immune response against various pathogenic bacteria, viruses, fungi, and parasites and hence frequently referred to as host defense peptides (HDPs) (Bahar and Ren, 2013; Diamond *et al.,* 2009). AMPs are short (6 to 100 amino acids) peptides, exhibiting a net positive charge and adopting amphipathic structures (Giuliani *et al.,* 2007; Hartmann *et al.,* 2010). HDPs can be categorized depending on their adopted structures into four subclasses: α-helical, flexible, β-sheet, and loop peptides (Lee *et al.,* 2015). The most predominant subgroup of AMPs is amphipathic α-helices such as maculatins, citropin, and melittin (Zhang *et al.,* 2016). AMPs offer promising activities to tackle infections produced by pathogens resistant to conventional antibiotics. These molecules display broad-spectrum activities against pathogenic microorganisms by interacting with and disrupting bacterial cell

*Corresponding Author
Mohammad Alsaggar, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. E-mail: mhalsaggar @ just.edu.jo*

membranes. Moreover, they can interfere with macromolecule synthesis like DNA and proteins (Kumar *et al.,* 2018).

Mounting research is currently directed towards AMPs design, aiming for the development of lead entities in antimicrobial drug discovery and overcoming limitations associated with naturally existing peptides, particularly toxicity, and spectrum of activity. Additionally, several combinations of selected AMP and conventional antibiotics have been proposed, showing synergistic effects as well as reduced drug toxicity (Almaaytah *et al.,* 2018). Mounting evidence suggests that AMPs influence the integrity of the bacterial membrane, thereby enhancing the permeability and sensitivity of bacterial strains towards antibiotics in combinations (Guilhelmelli *et al.,* 2013; Zhang *et al.,* 2014).

Brevinin-1E (FLPLLAGLAANFLPKIFCKITRKC) is a membrane-active agent that is isolated from the granular glands in the skins of the European frog *Rana* species (Zohrab *et al.,* 2019). Brevinin-1E is characterized by a broad range of activities against various bacterial strains. Also, it displays strong hemolytic activity against erythrocytes which hinders its application as a therapeutic antimicrobial agent (Rinaldi, 2002). On the other hand, bombolitin IV (INIKDILAKLVKVLGHV) is expressed in the *Megabombus pennsylvanicus* species, where it is secreted into the bumblebee venom (Roberson *et al.,* 2019). Similarly, it possesses a broad-spectrum antimicrobial that is accompanied by hemolytic effects that limit its use.

In this research, we designed a novel peptide that comprises merged active fragments of the brevinin-1E and bombolitin IV peptides to create a hybrid peptide named the SAMA peptide possessing increased antimicrobial effects and improved safety profile in comparison with the parent peptides. Our peptide SAMA displayed promising antimicrobial and antibiofilm activities when tested on a broad range of selected bacterial strains and importantly exhibited lower hemolytic activity compared to the parent peptides.

MATERIALS AND METHODS

Cell lines

Bacterial strains were obtained from the American Type Tissue Culture Collection (ATCC) and these involve two Gram-positive bacteria, methicillin-susceptible *Staphylococcus aureus* (ATCC 29215) and methicillin-resistant *S. aureus* (ATCC BAA-41). Additionally, two Gram-negative strains involving susceptible *Escherichia coli* (ATCC 25922) and the drug-resistant strain *E. coli* (ATCC BAA 2452) were also obtained. Adenocarcinoma human alveolar basal epithelial cells (A549) were also purchased from the ATCC (ATCC CCL-185).

Antibiotics

Levofloxacin, chloramphenicol, and doxycycline were purchased from Sigma-Aldrich, China. Ampicillin was obtained from Sigma-Aldrich, Canada. Rifampicin was obtained from Bio Basic, Canada. Stock solutions were made as per the manufacturer's instructions and stored at the recommended storage temperature for each antibiotic.

Synthesis and purification of SAMA

The SAMA peptide (NH$_2$-GRLIDKIARKLVKKIQRF ARKFF-COOH) was obtained from GL Biochem (Shanghai, China). The solid-phase methods and Fmoc chemistry were used to synthesize the peptide, followed by reverse-phase high performance liquid chromatography for peptide purification by 95.5%. Electrospray ionization mass spectrometry was used to verify the identity of the peptide.

Bioinformatic analysis

The estimated helicity of SAMA was predicted using the Network Protein Sequence Analysis software. On the other hand, the HeliQuest software was used to calculate SAMA hydrophobicity (H) and hydrophobic moment (µH). However, molecular weight, water solubility, isoelectric point, and net charge at neutral pH for the hybrid peptide were predicted using Innovagen's peptide calculator. Besides, Antimicrobial Peptide Calculator and Predictor tool in Antimicrobial Peptide Database (APD3) was used for the estimation of the protein-binding potential (Boman index) of the hybrid peptide. Model reliability confirmation and 3D structure prediction were carried out using the I-TASSER software. Server webpages were accessed in the period of 02/2020–09/2020.

Bacterial susceptibility assay

The antibacterial activity of the SAMA peptide was assessed by measuring the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the broth microdilution method outlined by the Clinical and Laboratory Standards Institute guidelines (Brown *et al.,* 2005). In brief, different strains of bacteria at a 10^6 CFU/ml concentration were prepared by diluting overnight cell culture using a fresh Mueller Hinton Broth. 50 µl of diluted bacterial suspension was mixed with 50 µl of different peptide concentrations in 96-well microtiter plates. The growth of bacteria was assessed by determining optical density at $\lambda = 600$ nm. MBC was determined by sampling of 10 µl from each well, spreading into Mueller Hinton Agar (Oxoid Ltd., Basingstoke, UK), and incubating at 37°C for 24 hours before reading the MBCs. The MBC was determined as the lowest concentration of the peptide that is needed to diminish the number of survival bacterial cells to <0.1% after 24 hours of incubation.

Antibiofilm activity

Attenuation of biofilm formation was examined as described previously (Luca *et al.,* 2013), using the Calgary Biofilm Device. Briefly, 150 µl of the bacterial inoculum was added to each well of the 96-microtiter plate. Lids containing pegs were placed into the 96-microtiter plate on which biofilm-forming cells can adhere and grow, followed by orbital shaking at 125 rpm and incubation at 37°C for 20 hours. Plates were next rinsed three times with 200 µl of phosphate-buffered saline to eliminate planktonic cells. Next, pegs-lids were moved into a "challenge 96-well microtiter plate" containing peptide solutions (200 µl) at different concentrations, incubated at 37°C for 2 hours, and then transferred into a recovery plate. The growth of recovered bacteria was examined by determining the absorbance of the plate at wavelength of 595 define the minimum biofilm eradication concentration (MBEC).

Synergistic checkerboard assay

Synergistic effects of combinations of SAMA and selected antibiotics (levofloxacin, chloramphenicol, doxycycline,

ampicillin, and rifampicin) were examined against four bacterial strains according to previous studies (Dundar and Otkun, 2010; Orhan *et al.*, 2005). Briefly, different antibiotic and peptide concentrations (25 µl each) were added to 50 µl of diluted bacterial suspension in 96-well plates. After incubation for 18 hours at 37°C, absorbance was measured at 600 nm. The fractional inhibitory concentrations (FICs) index was calculated according to the following equation:

$$\text{FIC index} = \frac{\text{MIC of SAMA peptide in combination}}{\text{MIC of SAMA peptide alone}} + \frac{\text{MIC of antibiotic in combination}}{\text{MIC of antibiotic alone}}$$

When the FIC index was ≤0.5, the combination was considered synergistic. However, it was considered additive when $0.5 \leq \text{FICs} \leq 1$, indifferent when $1 < \text{FICs} \leq 4$, and antagonistic when $4 < \text{FICs}$.

Hemolytic assay

2 ml of human blood (Sigma-Aldrich, St. Louis, MO) was added to 48 ml of phosphate-buffered saline to reach a final concentration of 4% red blood cell (RBC) and centrifuged for 5 minutes at 2,000 rpm. The process was repeated twice by aspirating the supernatant and replacing it with a fresh buffer. 2 ml of different peptide solution concentrations were then mixed with 2 ml of RBC suspension and then incubated for 60 minutes at 37°C. Triton X-100 (Santa Cruz Biotechnology, Dallas, TX) was used as a positive control. After incubation and gentle vortexing, 1 ml was transferred from each sample and then centrifuged at 2,000 rpm for 5 minutes and then 200 µl was transferred from each supernatant into a 96-well plate, where absorbance was measured at 450 nm. Hemolysis percentage was measured using the following equation:

$$\% \text{ hemolysis} = \frac{A - A_n}{A_p - A_n} \times 100,$$

where "A" is the absorbance value of the tested solutions, "A_n" is the absorbance value of the negative control, and "A_p" is the absorbance value of the positive control.

MTT assay

As described previously (Nosrati *et al.*, 2020), briefly, adenocarcinoma human alveolar basal epithelial cells (A549) were cultured in the F-12K Medium provided with 10% of fetal bovine serum and 1% of a penicillin-streptomycin solution at 37°C. Cells were seeded at a concentration of 7×10^3 cells/well in a 96-well plate and incubated overnight at 37°C under 5% CO_2 with different concentrations of the peptide (100, 80, 60, 40, 20, 10, 5, and 1 µM). Then, 10 µl MTT solution (5 mg/ml in serum-free media) was added to each well and then the plates were incubated again at 37°C, 5% CO_2, and humidity of 95% for 4 hours. Media were then aspired and 200 µl of dimethyl sulfoxide per well was added. Plates were shaken for 1 minute and absorbance was measured at wavelength 590 nm. The viability percentage was determined according to the following equation:

$$\% \text{ viability} = \frac{\text{sample abs}}{\text{negative control abs}}.$$

The IC_{50} was statistically estimated using the GraphPad Prism software (V8.4).

RESULTS

Peptide design and characterization

The SAMA peptide comprises 23 amino acids. The design started with the hybridization of the peptide region (14–24) of brevinin-1E to form the N-terminus of our peptide and the peptide region (2–11) of bombolitin IV to form its C-terminus. The *in silico* characterization of the hybrid peptide suggested the need for further sequence modification to optimize the physicochemical parameters essential for antimicrobial activity, such as helicity, hydrophobicity, and net charge. Therefore, a series of amino acid substitutions and additions were performed to optimize the parameters essential for antimicrobial activity as predicted by *in silico* characterization of derivatives (Supplementary Tables 1 and 2), until a final sequence of the SAMA peptide was obtained with satisfactory physicochemical characters (Tables 1 and 2).

The SAMA peptide displayed features essential for beneficial antimicrobial activity: higher helicity than parent peptides, a net charge of +8 at physiological pH, and intermediate level of hydrophobicity. The helical structure as predicted by the I-TASSER software (Zhang, 2008) of the SAMA peptide is depicted in Figure 1.

Supplementary Table 1. Predicted physicochemical parameters of parents' peptides and modified hybrid peptide derivatives.

Peptide	Peptide sequence	Length	Net charge	Hydrophobicity	Hydrophobic moment	Boman index	α-helix	Penetration	Bioactivity
Brevinin-1E	FLPLLAGLAANFLPKIFCKITRKC	24	4	0.759	0.316	−0.48	79.17	0.38	0.99
Bombolitin IV	INIKDILAKLVKVLGHV	17	2	—	—	−0.45	70.59	0.27	0.54
Hybrid peptide	PKIFCKITRKCIKDILAKLVK	21	6	0.526	0.531	0.88	71.43	0.39	0.95
	GPKIFCKITRKCIKDILAKLVK	22	6	0.524	0.531	0.8	68.18	0.37	0.94
	GKIFCKITRKCIKDILAKLVK	21	6	0.516	0.523	0.84	61.9	0.38	0.97
Brevinin-1E (14–24)	GKIFCKITRKCIKKILAKLVK	21	8	0.504	0.528	0.69	61.9	0.54	0.99
	GRIFCKITRKCIKKILARLVK	21	8	0.503	0.528	1.58	66.67	0.61	0.99
	GRIFKITRKIKKILARLVK	19	8	0.372	0.688	1.88	84.21	0.66	0.61
Bombolitin IV (2–11)	GRIFDKITRKIKKIQLARKLVK	22	8	0.185	0.377	2.52	86.36	0.69	0.41
	GRLIDKIARKLKKIQFARKFFK	22	8	0.199	0.374	2.46	86.36	0.61	0.46
SAMA peptide	GRLIDKIARKLVKKIQRFARKFF	23	8	0.262	0.664	2.58	91.3	0.644	0.76

Table 1. Sequences and estimated α-helical percentage of the parent peptides (brevinin-1E and bombolitin IV) and the SAMA peptide.

Peptides	Length	Net charge	Estimated pI	Molecular weight	B.I (kcal/mol)	% hydrophobicity
Brevinin-1E	24	+4	10.39	2,678.35	−0.48	99.9
Bombolitin IV	17	+2	10.45	1,873.33	−0.45	58
SAMA peptide	23	+8	12.17	2,832.48	2.58	47.83

Table 2. *In silico* characterization of the SAMA peptide compared with parent peptides.

Peptide	Amino acid sequences	α-helix (%)	Random coils (%)
Brevinin-1E	FLPLLAGLAANFLPKIFCKITRKC	79.17	20.83
Bombolitin IV	INIKDILAKLVKVLGHV	70.59	29.41
SAMA peptide	GRLIDKIARKLVKKIQRFARKFF	91.30	8.7

The net charge is peptide charge at physiologic pH. pI is the isoelectric point.
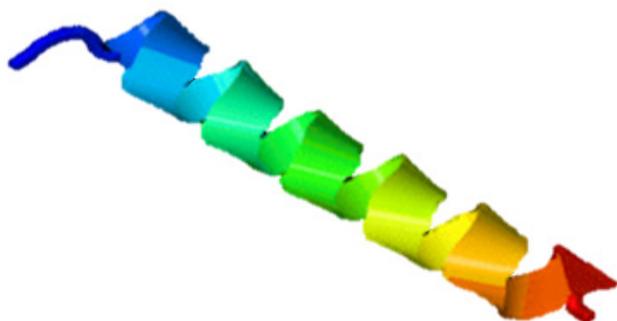
Figure 1. Predicted 3D structure of the SAMA hybrid peptide. The structure was created by the I-TASSER software.

Antimicrobial activity of SAMA

To evaluate the activity and the spectrum of SAMA's antimicrobial effects, four different strains of bacteria were used: a sensitive strain of Gram-positive *S. aureus* (ATCC 29215) and a multidrug-resistant *S. aureus* (ATCC BAA-41), along with sensitive and multi-drug-resistant strains of Gram-negative *E. coli* (ATCC 25922 and ATCC 2452, respectively). The SAMA peptide exhibits significant antimicrobial effects against Gram-positive strains, with MIC and MBC values of 5–15 µM (Table 3). Additionally, SAMA was more potent against Gram-negative strains with MIC and MBC values of 5 µM. Together, these results demonstrated a broad-spectrum bactericidal activity of the SAMA peptide.

Antibiofilm activity of SAMA

Biofilm formation is among the key bacterial virulent factors through which bacterial strains acquire their resistance to a broad array of antibiotics. Therefore, we investigated the potential attenuation of biofilm formation in bacteria upon SAMA treatment to determine the MBEC. Indeed, the recovery and growth of bacteria from SAMA-treated *S. aureus* (ATCC 29215) and *S. aureus* (ATCC BAA-41) biofilms were attenuated even after 8 hours of incubation in recovery media. While a peptide concentration up to 40 µM was sufficient to attenuate biofilm formation in Gram-positive strains (Table 4), biofilm-forming Gram-negative strains exhibited more resistance to SAMA activity, and concentrations up to 120 µM were not sufficient to attenuate biofilm formation.

Synergistic antimicrobial activity of SAMA

Given that modern therapeutics often relies on combined regimens that allow the use of lower doses, maximize the effects, and minimize drug toxicities, we next investigated potential additive or synergistic effects of SAMA when combined with selected antibiotics using the checkerboard microtiter plate assay. We determined MIC values of the antibiotics alone and then MICs of SAMA-antibiotic combinations. As summarized in Table 5, the calculated MICs of both SAMA and the selected drugs reduced dramatically in combinations with various percentages, and many

Table 3. MIC and MBC of the SAMA peptide against different bacterial strains.

	ATCC	MIC value (µM)	MBC value (µM)
Gram-positive strains			
S. aureus	29215	10	10
S. aureus	BAA-41	15	15
Gram-negative strains			
E. coli	25922	5	5
E. coli	BAA-2452	5	5

Table 4. MBEC of the SAMA peptide on different strains.

	ATCC	MBEC value (µM)
Gram-positive strains		
S. aureus	29215	30
S. aureus	BAA-41	40
Gram-negative strains		
E. coli	25922	>120
E. coli	BAA-2452	>120

Table 5. MICs of SAMA and antibiotics and the calculated FIC.

Bacterial species		Antibiotics			SAMA peptide			FIC index	
		MIC alone (µM)	MIC in combination (µM)	Reduction in MIC%	MIC alone (µM)	MIC in combination (µM)	Reduction in MIC%		
E. coli 25922	LVX	0.004	0.000125	96.9		1.25	75	0.28	S
	CHL	10	1	90		1.25	75	0.35	S
	RIF	10	1	90	5	1.25	75	0.35	S
	AMP	12.5	7.5	40		0.063	98.7	0.6126	A
	DOX	2	0.5	75		0.75	85	0.4	S
E. coli 2452	LVX	20	5	75		0.125	97.5	0.275	S
	CHL	20	5	75		1.25	75	0.5	S
	RIF	6	4.5	25	5	1.25	75	1	A
	AMP	1,750	1,750	---		0.063	98.7	1.0125	A
	DOX	10	4	60		0.063	98.7	0.4126	S
S. aureus 29215	LVX	0.22	0.066	70		0.2	98	0.32	S
	CHL	15	6	60		0.2	98	0.42	S
	RIF	5	1.25	75	10	0.5	95	0.3	S
	AMP	5	3.75	25		0.2	98	0.77	A
	DOX	2.5	0.25	90		4	60	0.5	S
S .aureus BAA-41	LVX	10	1	90		12	20	0.9	A
	CHL	30	3	90		6	60	0.5	S
	RIF	10.5	0.525	95	15	3	80	0.25	S
	AMP	1,500	825	45		6	60	0.95	A
	DOX	31.5	3.15	90		0.225	98.5	0.115	S

LVX, levofloxacin; CHL, chloramphenicol; RIF, rifampicin; AMP, ampicillin; DOX, doxycycline; S, synergistic; and A, additive.

combinations displayed synergistic rather than additive effects on the planktonic cells of the studied strains.

While the levofloxacin combination with SAMA displayed synergistic effects, with a more substantial reduction in MIC values against Gram-negative than Gram-positive strains, this combination exhibited additive effects against the methicillin-resistant Gram-positive strain, with an FIC score of 0.9. On the other hand, the chloramphenicol-SAMA combination displayed synergistic effects on all tested bacterial strains, with MICs reduction percentages ranging from 60% to 90%. Similarly, the doxycycline-SAMA combination displayed synergistic effects on tested strains, with various percentages of reductions in MICs. Importantly, both rifampicin and ampicillin showed neither additive nor synergistic effects on multi-drug-resistant *E. coli* strain, while their combinations with SAMA displayed synergistic and additive effects on the other strains, respectively. Together, these data demonstrated that the beneficial antimicrobial activity of SAMA could be further exploited to lower drug resistance rates commonly reported with other antibiotics.

Toxicity evaluation of SAMA

Many cationic peptides are known for their hemolytic toxicity on RBCs. Therefore, we next examined the safety profiles of the SAMA peptide at its reported MIC and MBC. The *in vitro* hemolytic assay showed minimal hemolytic activity of SAMA at concentrations up to 10 µM (Table 6). Incubation of RBCs with higher concentrations of SAMA resulted in increased hemolytic toxicity that approaches 35% with concentrations beyond 65

Table 6. SAMA hemolytic effects on human RBCs upon exposure time of 60 minutes at different concentrations.

Peptide concentration (µM)	Hemolysis (%)
2	1.2
5	3.5
10	7.3
18	20.1
34	33.4
65	34.2
124	39.2

µM. These results suggested a low toxicity profile of SAMA at therapeutic concentrations required for antimicrobial activity.

Potential anticancer activity of SAMA

Several AMP exhibited anticancer activity against various cancer cell lines. In this direction, we examined the potential anticancer activity of the SAMA peptide by examining its effects on the viability of adenocarcinomic human alveolar basal epithelial cells (A549). As shown in Figure 2, SAMA displayed potent antiproliferative effects on A549 cells, with an IC_{50} around 4.5 µM.

DISCUSSION

Antimicrobial resistance exemplifies one of the most challenging health problems nowadays. Therefore, it becomes imperative to direct efforts towards the development of antimicrobial therapies with improved safety and efficacy to tackle infections produced by drug-resistant strains (Aslam
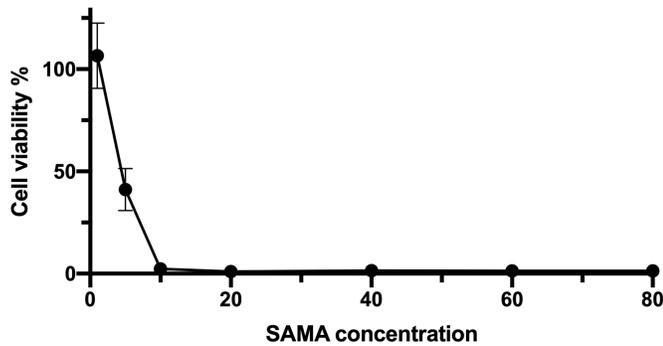
Figure 2. Growth curve of A549 cells after treatment with different concentrations (µM) of the SAMA peptide. Values represent the means (±SD) of six different experiments.

et al., 2018). AMPs have emerged as promising treatment options (Jenssen *et al.,* 2006), and mounting research is currently directed to designing and developing novel peptides for managing bacterial infections. Here, we designed and characterized a novel peptide derived from the sequences of two natural AMPs, which demonstrated promising antimicrobial and anticancer activities.

Hybridization is one of the peptidomimetics strategies used to reduce toxicity and enhance the activity of antimicrobials of AMPs (Wang *et al.,* 2015). This approach relies on combining the bioactive fragments of natural AMPs. The resulting hybrid peptide can be further modified with selected point mutations for improved physicochemical properties and enhanced biological activity. In this direction, we designed a hybrid peptide (PKIFCKITRKCIKDILAKLVK) comprising the bioactive fragments obtained from 14 to 24 of brevinin-1E and from 2 to 11 of bombolitin IV. Accumulated evidence suggests that the antimicrobial activity for peptides is predominantly a function of helicity content, hydrophobicity, and the net charge, wherein higher helicity, lesser than 50% hydrophobicity, and a cationic net charge (~ +7) are features of a biologically active peptide. Therefore, the sequence of the hybrid peptide was modified, and derivatives were *in silico* characterized (Supplementary Tables 1 and 2) to result in the SAMA peptide with a sequence of GRLIDKIARKLVKKIQRFARKFF. SAMA comprises 23 amino acids with an enhanced helicity percentage of 91.3% (Table 1; Fig. 1). The modified SAMA peptide contains nine positively charged residues including five lysine and four arginine residues,

with one aspartic acid residue contributing one negative charge, bringing the net charge to +8, which is sufficient for the initial electrostatic interaction with the negatively charged phospholipids on the bacterial membranes. SAMA also contains 11 hydrophobic residues (1 valine, 2 alanine, 2 leucine, 3 phenylalanine, and three isoleucine) with a total hydrophobic percentage of 47.83% with a hydrophobic momentum of 72% reflecting the amphipathicity of the peptide, which facilitated the antimicrobial activity, with minimal hemolytic toxicity. Several studies indicated that less than 50% hydrophobicity had been determined as an optimum value for AMPs to bind and disorder the integrity of bacterial membranes (Yin *et al.,* 2012), whereas a higher level of hydrophobicity results in a decrease in the antimicrobial specificity (Chen *et al.,* 2007). A high Boman index value indicates that SAMA would hit multi-intracellular targets in bacteria, owing to a high propensity for interaction with a wide range of proteins.

Upon *in vitro* functional characterization, the SAMA peptide effectively attenuated the growth of Gram-negative bacteria at an MIC of 5 µM. With a lesser potency, it inhibited the growth of Gram-positive bacteria within the range of 10–15 µM. The difference in the composition of the cell wall seems to affect the permeability of the SAMA peptide. Therefore, the interaction of the SAMA peptide with teichoic acids in Gram-positive bacteria may act as a trap for it on its way to the cytoplasmic membrane, resulting in a decreased peptide concentration on the inner membrane (Malanovic and Lohner, 2016). In addition, the MBC values of the SAMA peptide were similar to the MIC values, suggesting a bactericidal antimicrobial activity. In addition to microbial growth inhibition, we examined the antibiofilm activity of the SAMA peptide, which was solely efficient against Gram-positive strains, but not Gram-negative ones. These findings could be explained by the unique biochemistry of the *E. coli* outer membranes. It has been reported that *E. coli* secretes an anionic polysaccharide identified as alginate, which in turn can interact with cationic AMPs, resulting in the capture and the neutralization of these peptides and subsequent protection of *E. coli* in the biofilm from the bactericidal actions of positively charged AMPs (Yasir *et al.,* 2018). Moreover, Gram-negative bacteria such as *E. coli* have a protective genetic system to add aminoarabinose to lipid A that results in decreasing the outer membrane net negative charge. Indeed, it was shown that modification of lipid A attenuated the electrostatic attraction of cationic AMPs toward the Gram-negative bacteria (Band and Weiss, 2015).

Supplementary Table 2. Amino acid sequences of the modified hybrid peptide and the reason for the modification.

Derivatives	Rationale for modification
GPKIFCKITRKCIKDILAKLVK	Based on the N-end rule, the N-terminal residue plays a significant role in determining its stability (Yeom *et al.,* 2017).
GKIFCKITRKCIKDILAKLVK	The *percent* of *helicity* increased as the number of *proline* amino acids *decreased*. (Morgan and Rubenstein, 2013).
GKIFCKITRKCIKKILAKLVK	Negatively charged aspartic acid was replaced by positively charged lysine to increase the net positive charge of the peptide.
GRIFCKITRKCIKKILARLVK	Arginine is more basic than lysine due to having a guanidine group and helps in more permeabilization (Cutrona *et al.,* 2015).
GRIFKITRKIKKILARLVK	Cysteine amino acid in primary sequence form disulfide bonds in secondary structure. The *percent* of *helicity* increased as the number of disulfide bonds *decreased*, (Marques *et al.,* 2010), so cysteines were deleted.
GRIFDKITRKIKKIQLARKLVK	The *percent* of *hydrophobicity decreased* as the number of polar amino acids increased.
GRLIDKIARKLKKIQFARKFFK	To increase the hydrophobic moment value.
GRLIDKIARKLVKKIQRFARKFF	To increase the hydrophobic moment value and Boman index.

Different antibiotic combinations were evaluated to reduce the toxicity and improve the antibacterial activity of both the SAMA peptide and individual conventional antibiotics. All tested antibiotics are active intracellularly except ampicillin, which inhibits bacterial growth by targeting the bacterial cell wall. Therefore, the combination of the SAMA peptide with ampicillin displays no synergistic effects, because of the different sites of actions of the two peptides. Other combinations, however, showed synergistic or at least additive effects. Based on the mechanism of action that is suggested for the SAMA peptide, it may affect the integrity of the bacterial membrane through pore formation resulting in enhancing the permeability of bacterial cells and increasing influx and uptake of antibiotics into the cells to accomplish the destruction process. As a result, more intracellular accumulation of antibiotics is expected and hence the increased sensitivity of the bacterial strains, which are normally resistant to conventional antibiotics.

Parallel to functional characterization, the SAMA peptide toxicity was investigated for potential hemolytic activity against RBCs. Hemolysis is a common side effect for cationic peptides and is usually linked to peptide hydrophobicity and amphipathicity. At therapeutic concentrations, SAMA displayed minimal hemolytic activity of lesser than 10% and demonstrated a safer profile compared to the parent peptides (brevinin-1E and bombolitin IV), which exhibited significant hemolytic effects at even lower concentrations (Son *et al.,* 2003; Waghu *et al.,* 2016). This could be attributed to the more abundant polar amino acids in the SAMA N-terminus that could contribute to breaking the nonpolar side of the helix upon binding the RBC membrane, the feature that is deemed essential for peptide-mediated hemolytic activity.

Several studies have demonstrated that AMPs possess promising anticancer activity against several cancer cell types. Importantly, the antiproliferative activity was selective for cancer cells over the normal cell (Papo and Shai, 2005). Here, the anticancer activity of the SAMA peptide was examined against adenocarcinomic human alveolar basal epithelial cells. SAMA proved to be a potential anticancer agent against A549 cells (Fig. 2). This could be explained by the mechanism suggested for melittin and the defensins against cancer cells (Hanaoka *et al.,* 2016; Moreno and Giralt, 2015). These peptides bind to the cancer cell membrane, destabilizing it, causing their necrotic death. Mounting evidence suggests that these peptides would display selectivity toward cancerous cells, as the cytoplasmic membrane of cancer cells contains more abundant negatively charged phosphatidylserine in addition to O-glycosylated mucins (Deslouches and Di, 2017). Another explanation is the presence of a high percentage of microvilli on cancerous cells relative to the normal cells and, hence, increased surface area on cancerous cells and increased accumulation and binding of the peptide on cell surface (Papo and Shai, 2005).

CONCLUSION

In summary, we have designed and characterized a modified hybrid peptide using the sequences of brevinin-1E and bombolitin IV. SAMA exhibited improved antimicrobial effects on planktonic Gram-negative and Gram-positive bacterial cells with a promising safety profile. SAMA displayed a low hemolytic effect against mammalian erythrocytes. Furthermore, lower MICs and hence improved safety for SAMA were achieved upon the combination with five kinds of conventional antibiotics. In addition, SAMA exhibited antibiofilm activities, albeit at higher concentrations, extending the use of peptides for nonautoclavable inanimate surfaces.

AUTHORS' CONTRIBUTIONS

M. Alsaggar and Y. Al Tall conceptualized the study. A. Al-Tarawneh and M. Al-Hazabreh conducted the methodology. M. Alsaggar and A. Al-Tarawneh carried out the analysis and investigation. M. Alsaggar and Y Al Tall wrote and edited the manuscript. M. Alsaggar acquired the funding. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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