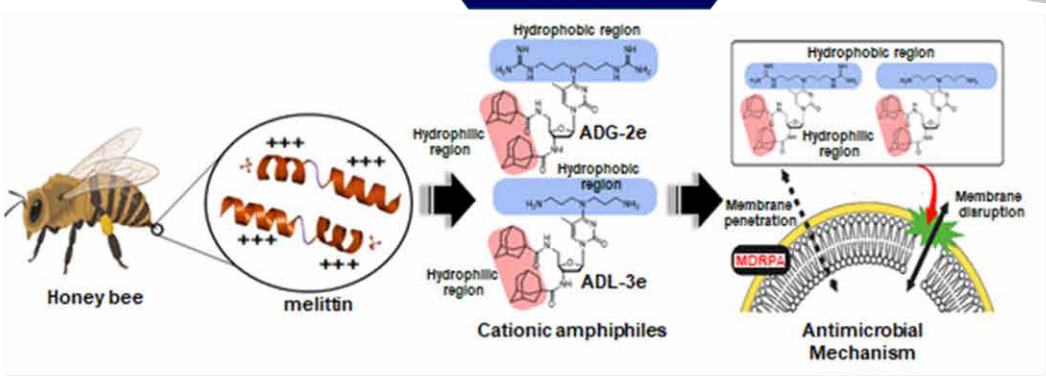
Deoxythymidine-based antimicrobial peptidomimetics to combat drug-resistance

https://doi.org/10.17952/37EPS.2024.P1124

Eun Young Kim[1], Dinesh Kumar[1], Sung Tae Yang[2] and Song Yub Shin[1,*]

[1]Department of Cellular & Molecular Medicine, School of Medicine, Chosun University, Gwangju, 61452, Republic of Korea
[2]Department of Department of Microbiology, School of Medicine, Chosun University, Gwangju, 61452, Republic of Korea

Abstract



Deoxythymidine-based amphiphilic antimicrobial peptidomimetics, ADG-2e and ADL-3e, were assessed for their potential as novel classes of antimicrobial, antibiofilm, and anti-inflammatory agents. ADG-2e and ADL-3e demonstrated good resistance to physiological salts and human serum, with a low incidence of drug resistance. They also exhibit proteolytic resistance against pepsin, trypsin, α-chymotrypsin, and proteinase K. ADG-2e and ADL-3e were found to kill bacteria by an intracellular target mechanism and a bacterial cell membrane-disrupting mechanism, respectively. Furthermore, when combined with several conventional antibiotics against MRSA and MDRPA ADG-2e and ADL-3e showed effective synergistic effects. Importantly, ADG-2e and ADL-3e not only suppressed MDRPA biofilm formation but also effectively eradicated mature MDRPA biofilms. Additionally, ADG-2e and ADL-3e significantly reduced TNF-α and IL-6 gene expression and protein secretion in LPS-stimulated macrophages, indicating potent anti-inflammatory activity during LPS-induced inflammation. Our findings suggest that ADG-2e and ADL-3e have the potential to be further developed as novel antimicrobial, antibiofilm, and anti-inflammatory agents for combating bacterial infections.

Drug Resistance & Time-Killing Kinetic

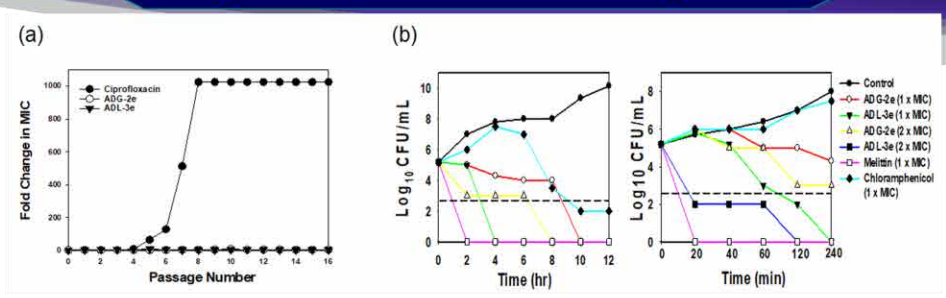


Fig. 3. (a) The emergence of drug resistance after treatment of *S. aureus* (KCTC 1621) with ADG-2e, ADL-3e, and ciprofloxacin for 16 passages at a sub-MIC concentration determined by the sequential passaging method. The fold change in MIC of the antimicrobials (compounds) was plotted against the number of passages. (b) Time-killing kinetic analysis of ADG-2e and ADL-3e against *E. coli* (KCTC 1682). The dotted line indicates a 50% reduction from the initial CFU.

Antimicrobial Mechanism

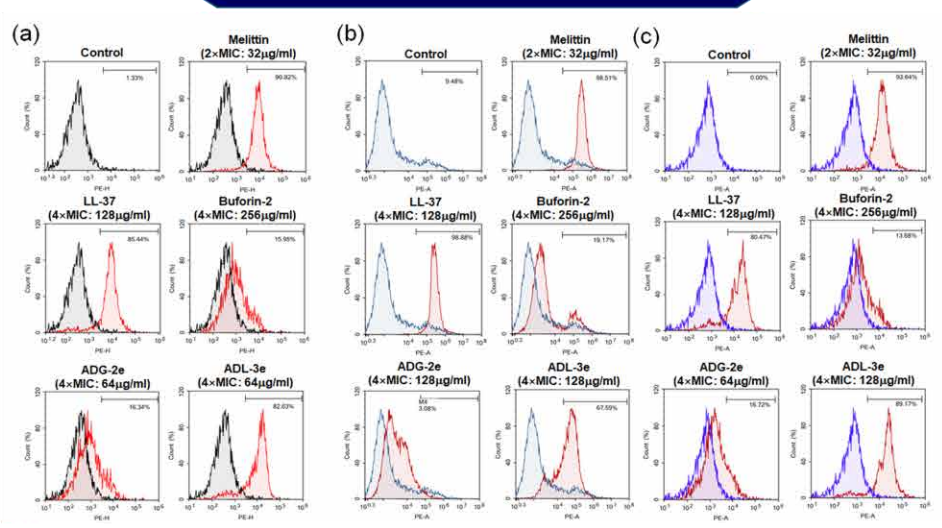


Fig. 4. Flow cytometric analysis of *E. coli* (KCTC 1682) (a), MRSA (CCARM 3089) (b) and MDRPA (CCARM 2095) (c) treated with 2xMIC or 4xMIC of ADG-2e, ADL-3e and control peptides (melittin, buforin-2 and LL-37) for 60 min and stained with PI.

Structure & Protease Stability

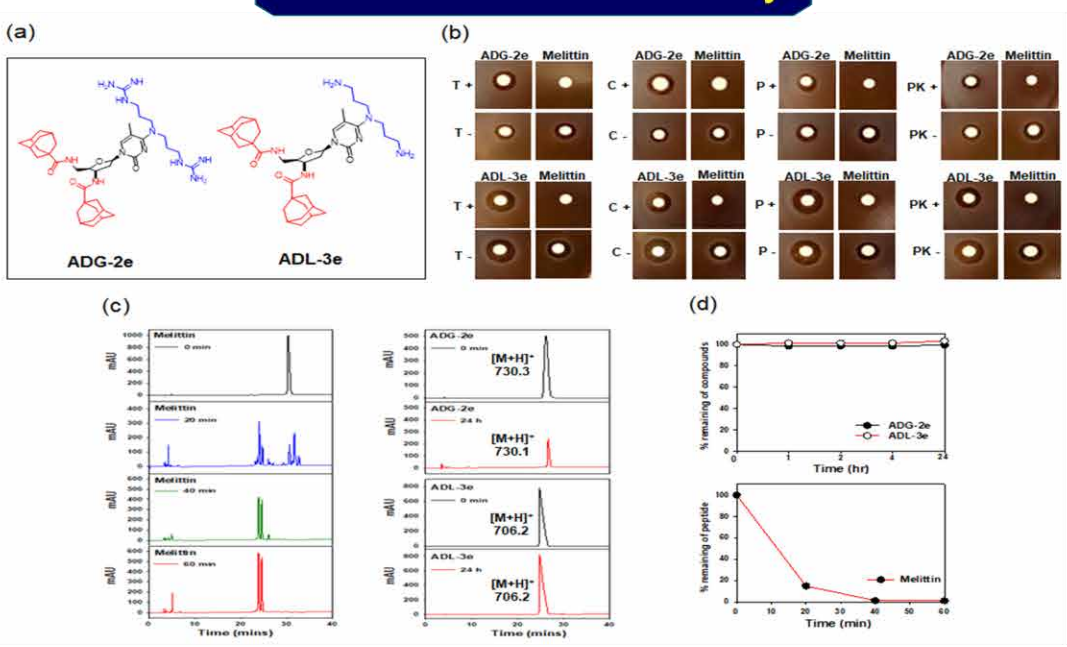


Fig. 1. (a) Chemical structures of deoxythymidine-based cationic amphiphiles, ADG-2e and ADL-3e. The blue and red colors indicated the cationic and hydrophobic moiety of amphiphiles, respectively. (b) Effect of protease on the bactericidal activity of ADG-2e and ADL-3e determined by radial diffusion assay. Compounds were incubated with pepsin, trypsin, α-chymotrypsin, or proteinase K for 4 h at 37°C. Subsequently, 20ul of the enzyme-treated compounds were added into wells in the *E. coli* (KCTC 1682) (10^5-10^6 CFU/ml) mixed MH-broth agar plate and incubated overnight at 37°C. T+: with trypsin, T-: without trypsin, C+: with α-chymotrypsin, C-: without α-chymotrypsin, P+: with pepsin, P-: without pepsin, PK+: with proteinase K, PK-: without proteinase K. (c) Reverse-phase high-performance liquid chromatography (RP-HPLC) profiles of tryptic digestion products of melittin, ADG-2e and ADL-3e obtained at varied intervals. The main eluents of trypsin-treated ADG-2e and ADL-3e for 24 h were collected, lyophilized and analyzed by electrospray ionization-mass spectroscopy (ESI-MS). (d) Stability of melittin, ADG-2e and ADL-3e to trypsin digestion.

Table 1. The MIC values of ADG-2e, ADL-3e and conventional antibiotics against MRSA, MDRPA and VREF

Bacterial strains	MIC (ug/ml)					
	ADG-2e	ADL-3e	CHL	TET	RIF	CIP
MRSA[a]						
CCARM 3001	32	16	512	64	32	128
CCARM 3089	16	16	1024	128	32	1024
CCARM 3090	16	16	1024	128	32	512
CCARM 3095	16	32	2048	256	8	1024
MDRPA[b]						
CCARM 2095	32	32	2048	256	16	1024
CCARM 2109	32	16	1024	128	32	512
VREF[c]						
ATCC 515675	32	32	128	256	64	128
ATCC 51559	16	16	1024	>2048	32	128

[a]MRSA: Methicillin-resistant *Staphylococcus aureus* [b]MDRPA: Multidrug-resistant *Pseudomonas aeruginosa*
[c]VREF: Vancomycin-resistant *Enterococcus faecalis* CHL: Chloramphenicol, TET: Tetracycline, RIF: Rifampicin, CIP: Ciprofloxacin

Table 2. The MIC values of ADG-2e, ADL-3e and antibiotics in the presence of physiological salts and human serum

Compounds	Control	NaCl 150 mM	KCl 4.5 mM	NH_4Cl 6 uM	$MgCl_2$ 1 mM	$CaCl_2$ 2.5 mM	$FeCl_3$ 4 uM	Human serum (25%)
MRSA (CCARM 3089)								
ADG-2e	16	16	16	16	16	16	32	32
ADL-3e	32	32	32	32	32	32	32	32
CHL	2048	2048	2048	2048	2048	2048	2048	2048
TET	256	256	256	512	512	256	512	512
RIF	8	16	16	8	8	8	8	16
CIP	1024	1024	1024	1024	1024	1024	1024	1024
MDRPA (CCARM 2095)								
ADG-2e	32	32	32	16	16	32	32	16
ADL-3e	32	32	32	32	32	32	32	32
CHL	2048	2048	2048	2048	2048	2048	2048	2048
TET	256	256	512	256	256	512	512	1024
RIF	16	32	16	16	16	32	32	32
CIP	1024	1024	1024	2048	2048	2048	2048	1024

Antimicrobial Mechanism

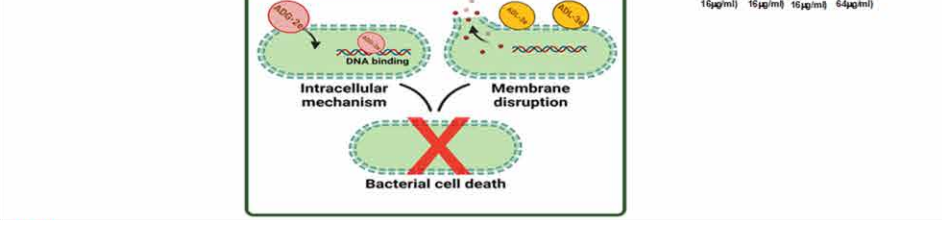


Fig. 5. Scanning electron microscopy (SEM) images of *E. coli* (KCTC 1682) (a) and MRSA (CCARM 3089) (b). Mid-logarithmic-phase bacteria cells were treated with compound at 1xMIC, 2xMIC and 4xMIC for 4 h. The control was done without compound. Scale bar: 200 nm.

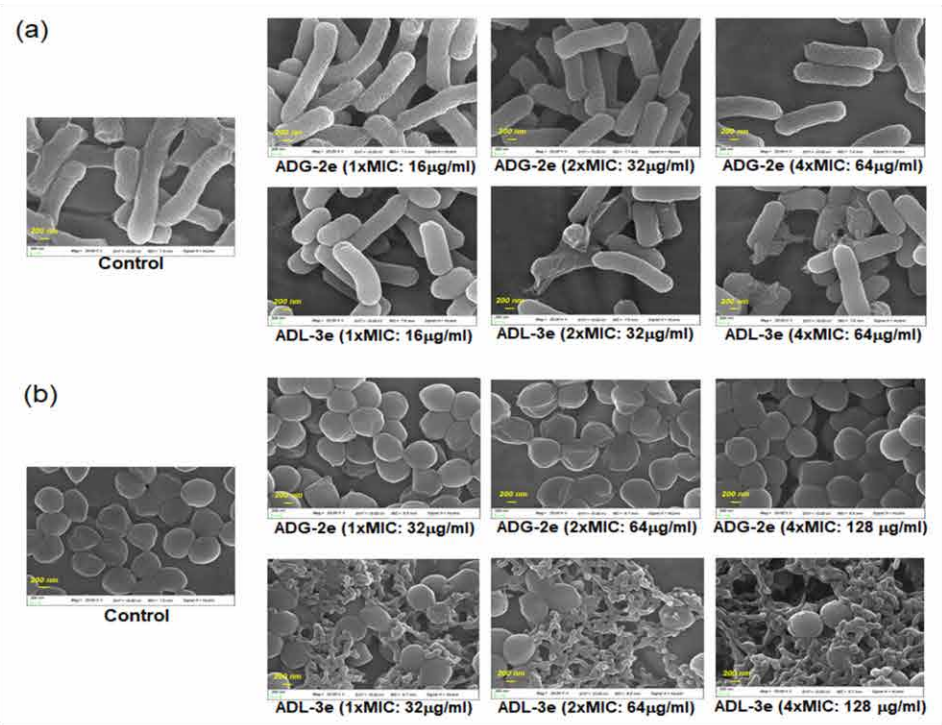


Fig. 6. (a) Interaction of compounds with *E. coli* (ATCC 25922) genomic DNA by a gel migration assay. Binding was assayed by measuring the degree of inhibition of migration by *E. coli* (ATCC 25922) genomic DNA (500 ng). DNA and compounds were co-incubated for 1 h at room temperature before electrophoresis on a 1.0% agarose gel. Line 1-8: The concentration of compounds was 0, 2, 4, 8, 16, 32, 64, and 128ug/ml, respectively. M: DNA marker (b) Inhibitory effect of *E. coli* (ATCC 25922) genomic DNA on antimicrobial activity of ADG-2e, ADL-3e, and control AMPs (melittin and buforin-2). The values are expressed as the mean ± SEM of three independent experiments and are statistically significant at $^{**}p < 0.01$. (c) Schematic diagram showing the potential antimicrobial mechanism of ADG-2e and ADL-3e

Synergy Activity & Antibiofilm Activity

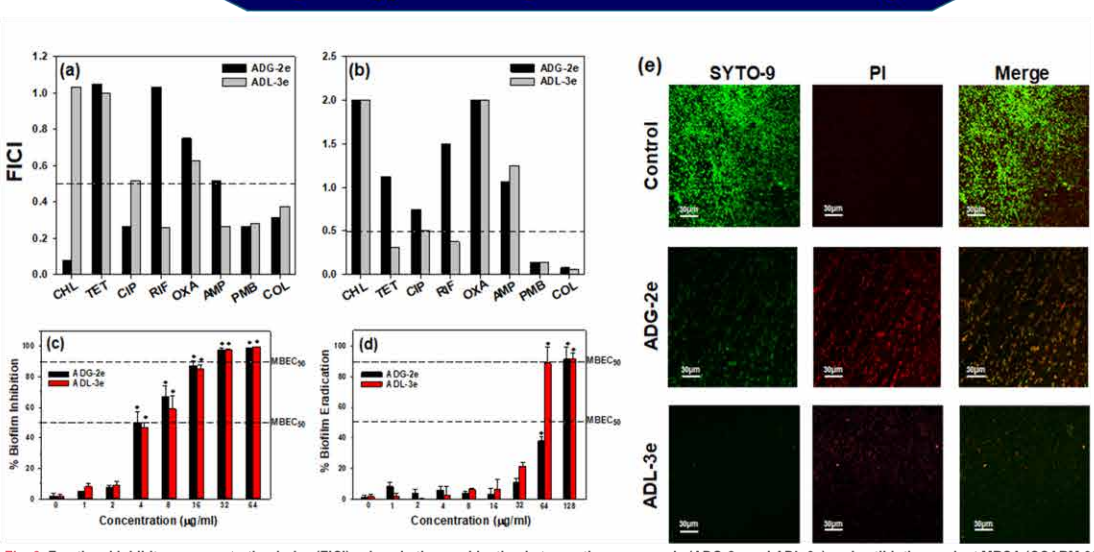


Fig. 2. Fractional inhibitory concentration index (FICI) values in the combination between the compounds (ADG-2e and ADL-3e) and antibiotics against MRSA (CCARM 3089) (a) and MDRPA (CCARM 2095) (b). The dotted line represents FICI=0.5. FICI ≤ 0.5 = synergy. In vitro antibiofilm activity of compounds. Biofilm inhibition (c) and eradication (d) activities of ADG-2e and ADL-3e against MDRPA (CCARM 2095). The values are expressed as the mean ± SEM of three independent experiments and are statistically significant at $^{*}p < 0.05$. (e) Confocal laser scanning microscopy (CLSM) images of compound-treated mature MDRPA biofilms. Biofilms were 24 h and then treated with PBS solution (control), ADG-2e ($MIEC_{90}$) and ADL-3e ($MIEC_{90}$) for 2h. Biofilms were stained with the live (green) and dead (red) stain kit, which uses SYTO-9 to stain live cells and propidium iodide (PI) to stain dead cells. The scale bar represents 30 um.

Anti-inflammatory Activity

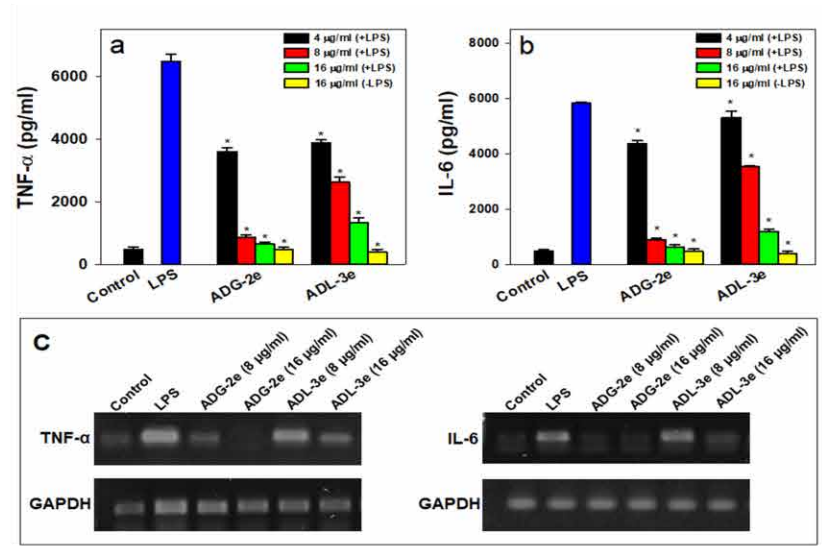


Fig. 7. ADG-2e and ADL-3e suppressed LPS-induced production and expression of TNF-alpha and IL-6 in LPS-stimulated RAW264.7 cells. (a) Effects of ADG-2e and ADL-3e on TNF-alpha production in LPS-stimulated RAW264.7 cells. (b) Effects of ADG-2e, ADL-3e, and LL-37 on IL-6 production in LPS-stimulated RAW264.7 cells. All bar graphs represent mean ± SEM from three independent experiments ($^{*}P < 0.05$, significantly different from the control group treated with LPS alone). (c) Effects of ADG-2e and ADL-3e on the mRNA levels of TNF-alpha and IL-6 in LPS-stimulated RAW264.7 cells. RAW264.7 cells ($5x10^5$ cells/well) were incubated with the compounds in the presence of LPS (20 ng/mL) for 3 h. Total RNA was isolated and analyzed to determine the TNF-alpha and IL-6 mRNAs levels by RT-PCR.