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In-vitro effect of human cathelicidin antimicrobial peptide LL-37 on dengue virus type 2

K. Alagarasu[a,*], P.S. Patil[a], P. Shil[b], M. Seervi[a], M.B. Kakade[a], H. Tillu[a], A. Salunke[a]

[a] Dengue/Chikungunya Group, ICMR-National Institute of Virology, 20A, Dr. Ambedkar Road, Pune 411001, Maharashtra, India
[b] Bioinformatics Group, Microbial Containment Complex, ICMR-National Institute of Virology, Sus Road, Pashan, Pune 411021, Maharashtra, India

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ABSTRACT

Human Cathelicidin antimicrobial peptide LL-37 is known to have antiviral activity against many viruses. In the present study, we investigated the *in-vitro* effect of LL-37 on dengue virus type 2 (DENV-2) infection and replication in Vero E6 cells. To study the effect of pretreatment of virus or cells with LL-37, the virus was pretreated with different concentrations of LL-37 (2.5 μM–15 μM) or scrambled (Scr) LL-37(5 μM–15 μM) and used for infection or the cells were first treated with LL-37 and infected. To study the effect of LL-37 post infection (PI), the cells were infected first followed by addition of LL-37 to the culture medium 24 h after infection. In all conditions, after the incubation, the culture supernatant was assessed for viral RNA copy number by real time RT-PCR, infectious virus particles by focus forming unit assay (FFU) and non structural protein 1 (NS1) antigen levels by ELISA. Percentage of infection was assessed using immunoflourescence assay (IFA). The results revealed that pretreatment of virus with 10–15 μM LL-37 significantly reduced its infectivity as compared to virus control ($P < 0.0001$). Moreover, pretreatment of virus with 10–15 μM LL-37 significantly reduced the levels of viral genomic RNA and NS1 antigen ($P < 0.0001$). Treatment of virus with 10–15 μM LL-37 resulted in two to three log reduction of mean $\log_{10}$ FFU/ml as compared to virus control ($P < 0.0001$). Treatment of the virus with scrambled LL-37 had no effect on percentage of infection and viral load as compared to virus control cultures ($P > 0.05$). Pretreatment of cells before infection or addition of LL-37 to the culture 24 h PI had no effect on viral load. Molecular docking studies revealed possible binding of LL-37 to both the units of DENV envelope (E) protein dimer. Together, the *in-vitro* experiments and *in-silico* analyses suggest that LL-37 inhibits DENV-2 at the stage of entry into the cells by binding to the E protein. The results might have implications for prophylaxis against DENV infections and need further *in-vivo* studies.

1. Introduction

Dengue virus (DENV) serotypes 1–4 belonging to genus flavivirus and family of flaviviridae are the causative agents of mild dengue fever or occasionally fatal severe dengue which is characterized by vascular leakage symptoms. The virus is transmitted in humans by the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes. The world is witnessing an increase in the number of dengue cases every year which poses a great threat to the public health systems in developing countries [1]. A vaccine for dengue is approved in some endemic nations for use in individuals of nine to 45 years old. However, efficacy of the vaccine depends on the seroprevalence of dengue in the target population [2]. Till date, no antivirals have been approved for use against dengue and the search for an effective antiviral drug is still on.

Naturally occurring cationic peptides with antimicrobial activity from biological systems are being considered as possible drugs against pathogenic microbes and are being tested for their antimicrobial potential [3]. Among the human antimicrobial peptides, cathelicidin antimicrobial peptide LL-37 has received considerable attention due to its antimicrobial activity against diverse pathogens [4]. LL-37 is the only cathelicidin antimicrobial peptide found in humans. LL-37 is a 37 amino acid length peptide and derived from the cathelicidin precursor protein, also called human cathelicidin antimicrobial protein 18 (hCAP18), by the action of proteinase 3 [5]. In the skin, LL-37 is further cleaved in to shorter peptides with potent antimicrobial activity by the serine proteases stratum corneum tryptic enzyme (SCTE, Kallikrein 5) and stratum corneum chymotryptic protease (SCTE, Kallikrein 7) [6]. LL-37 has both antimicrobial as well as immunomodulatory activities. Antiviral activity of LL-37 has been reported against HIV-1, influenza A virus (IAV), respiratory syncytial virus (RSV), rhino virus, vaccinica virus, herpes simplex virus, hepatitis C virus and aichi virus [7–15]. LL-37 enhances the uptake of TLR3 ligands into keratinocytes and

* Corresponding author.
E-mail addresses: alagarasu@gmail.com, alagarasu.k@nic.in (K. Alagarasu).

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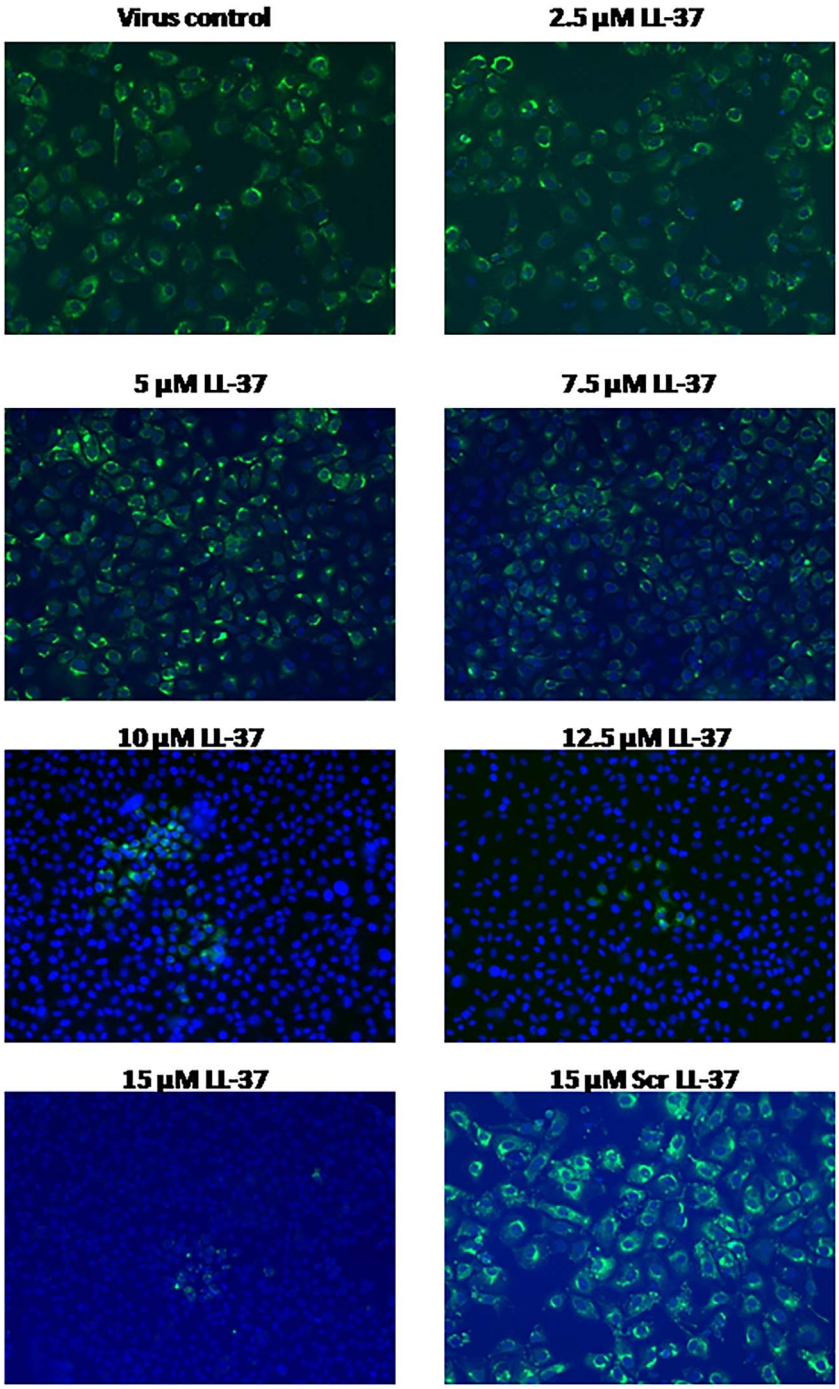

Fig. 1. Microscopic pictures of Vero E6 cells infected with virus pretreated with different concentrations of LL-37 and scrambled (Scr) LL-37 (20X magnification). Infected cells appear green (due to FITC tagged with antibody used) and uninfected cells appear blue (due to nuclear stain DAPI). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

augments the expression of IFN-β and subsequent antiviral activity [16]. LL-37 has been reported to augment the neutrophil H_2O_2 generation in response to IAV and also inhibited the production of proinflammatory cytokines [17].

DENV infection induces the expression of LL-37 in monocytic cells and neutrophils [18]. While transmitting the virus, saliva of *Aedes* mosquitoes counteracts the immune response in host by suppressing the expression of antimicrobial peptides and enhances viral infection. A 34 kDa salivary protein of *Aedes aegypti* was found to suppress the expression of LL-37 in human keratinocytes and enhance the replication of DENV [19]. These studies suggest a role for LL-37 in the immune response against DENV. However, the effect of LL-37 on DENV has not been studied. The aim of the present study was to investigate the *in-vitro* effect of LL-37 on DENV infectivity and replication in Vero E6 cells.

2. Materials and methods

2.1. Cell lines, virus strain, antibodies and reagents

Vero E6 (ATCC CRL-1586) and *Aedes albopictus* clone C6/36 (ATCC_CRL-1660) cell lines were obtained from ATCC. Dengue virus type 2 (DENV-2) (Indian strain 803347 from the repository of ICMR-National Institute of Virology, Pune, India) was used in all experiments. Both cell lines were maintained in minimal essential medium (MEM) (Gibco, Life