pISSN 2508-1357, eISSN 2508-139X
J Biomed Transl Res 2016;17(3):075-078
http://dx.doi.org/10.12729/jbtr.2016.17.3.075
Received 1 Sep. 2016, Revised 13 Sep. 2016, Accepted 13 Sep. 2016

Short communication

Phospholipase A2 isolated from the venom of honey bees prevents viral attachment in mammalian cells

C.Y. Hewawaduge[1†], Byeong-Hoon Lee[1†], Tae-Hwan Kim[1], Md Bashir Uddin[1,2], Jae-Hoon Kim[1], Choul Goo Kim[3], Jong-Soo Lee[1*]

[1]*College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Korea*
[2]*Sylhet Agricultural University, Sylhet, Bangladesh*
[3]*Chung Jin Biotech Corporation, Ansan 15577, Korea*
[†]*These authors contributed equally to this study*

The belief that honey bee venom (BV) can be used to treat certain immune-related diseases, such as arthritis and rheumatic conditions, goes back to antiquity. A growing number of reports have demonstrated that BV contains at least 18 pharmacologically active components, including phospholipase A_2 (PLA_2). Recent research has shown that bee venom PLA_2 ($bvPLA_2$) induces protective immune responses against several diseases including asthma, Parkinson's disease, and drug-induced organ inflammation. However, the antiviral properties of $bvPLA_2$ have not been well investigated. Hence, we examined the potential inhibitory effects of $bvPLA_2$ and its possible mechanism of action against a broad panel of pathogenic viruses *in vitro*. Pre-treatment with $bvPLA_2$ significantly inhibited the replication of vesicular stomatitis virus (VSV), coxsackie virus (H3), enterovirus-71 (EV-71), herpes simplex virus (HSV) and Adenovirus (AdV) dramatically. However, $bvPLA_2$ did not show antiviral activity against Influenza A virus (PR8) and Newcastle disease virus (NDV). Such inhibitory effects were explained by blocking of the attachment of the virus to cells upon $bvPLA_2$ treatment. Additionally, we observed that Heparan sulfate (HS) has an inhibitory effect on the attachment of HSV to the cell surface dose dependently, which was inconsistent with $bvPLA_2$ treatment. These findings suggest that $bvPLA_2$ has an inhibitory effect on the replication of diverse viruses by blocking their attachment to the cell surface and could be a promising source of natural antiviral agents.

Key words: bee venom phospholipase A_2, attachment assay, virus replication, inhibitory effects, heparan sulfate

The common honey bee belongs to the species *Apis mellifera* L. and participates in some activities that are closely related to human beings. Since ancient times, it has been believed that the honey bee venom (BV) can be used to treat certain immune-related diseases, such as arthritis and rheumatic conditions [1-3]. BV comprises several components, including cell-lytic peptides, diverse enzymes, and bioactive amines. The two major proteinaceous components of BV are melittin, a 26-amino acid cationic cell-lytic peptide, and phospholipase A_2 (PLA_2), an enzyme that hydrolyzes membrane phospholipids to produce lysophospholipids and arachidonic acid [4]. PLA_2 isolated from BV ($bvPLA_2$) belongs to the two notable families with a common enzymatic activity. It is well known that PLA_2 can induce an allergic response upon entering the body through bee or wasp stings [5]. However, PLA_2 also displays antibacterial and anticoagulant actions and plays an active role in anti-inflammatory processes [6-8]. Recently, it was revealed that PLA_2 has protective immune responses against several diseases including asthma, Parkinson's disease, Alzheimer's disease, and drug-induced organ inflammation [9,10]. Moreover, $bvPLA_2$ is sensed by the innate immune system and induces a receptor ST2-dependent primary type 2 immune response [11]. And also, PLA_2, which is isolated from the venom of *Crotalus durissus terrificus*, inactivates Dengue and yellow fever viruses, as well as other enveloped viruses by disrupting the viral envelope [12]. However, the underlying mechanisms of antiviral activities by $bvPLA_2$ have not been fully understood. Therefore, we investigated whether $bvPLA_2$ inhibits viral replication by other specific mechanism.

The PLA_2 was isolated from BV (Chung Jin Biotech Corporation, Korea) using high performance liquid chromatography (HPLC) instrument consisting of Waters

***Corresponding author:** Jong-Soo Lee,
College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Korea
Tel: +82-42-821-6753, Fax: +82-42-825-7910, E-mail: jongsool@cnu.ac.kr

2525u binary HPLC pump, Waters 996 photodiode array detector, Waters 717 plus and Waters Fraction Collector III. Chromatography was conducted on a Phenomenex Jupiter 10u Preteo 90A Column (250 X 21.20 mm), and 1,000 µL (10 mg/mL) of BV was injected. The mobile phase consisted of 0.2% TFA in water (Solvent A) and 0.22% TFA in acetonitrile (Solvent B). The gradient mode was as follows: 0 min 100% A and 0% B; 25 min 50% A and 50% B; 60 min 100% A and 0% B. The flow rate was 8.0 mL/min, and the elution was monitored at 220nm. Collected fractions were compared with standard PLA_2.

The inhibitory activity of $bvPLA_2$ was tested in HEK293T, HeLa, Vero, and MDCK cells (12 well plates: 5×10^5 cells/well) with diverse RNA and DNA viruses which can express the green fluorescent protein (GFP) by using the attachment assay. Briefly, for the attachment assay, viruses and $bvPLA_2$ (2.0 or 3.0 µg/mL) were simultaneously added into the cells and incubated with the cells for 1 hr at 4ºC. After washing, the cells were transferred to 37ºC. At 24 hour post infection (hpi), cells were harvested to measure the GFP absorbance (the cytopathic effect for EV-71) (data not shown), and supernatants (with and without cells) were collected for a standard plaque assay of the viruses (Fig. 1).

As shown in Fig 1, $bvPLA_2$ inhibited the replication of Vesicular Stomatitis virus (VSV-GFP, Fig.1A), Coxsackie virus (H3-GFP, Fig.1B), Enterovirus-71 (EV-71, Fig.1C), Herpes Simplex virus (HSV-GFP, Fig.1D) and Adenovirus (AdV-GFP, Fig.1E). However, $bvPLA_2$ couldn't inhibit the replication of Influenza A virus (PR8-GFP, Fig.1F) and Newcastle disease virus (NDV-GFP, Fig.1G). These results suggested that $bvPLA_2$ can block the attachment of diverse virus using specific cell receptors.

The binding of viruses to their target cells is the initial step in establishing an infection. Numerous viruses utilize specific or non-specific cellular receptors to infect target cells. A full spectrum of cell surface molecules, including glycoproteins, glycolipids, and proteoglycans participate in cell–virion interaction and recognition. Recent studies have revealed that heparan sulfate (HS) (Sigma, Korea) plays a role in facilitating viral infection through an interaction with viral proteins [13]. HS glycosaminoglycans also serve as receptors for the infection of Herpes Simplex virus (HSV), Adenovirus (Adv), Respiratory Syncytial virus (RSV), and Hand, Foot and Mouth disease virus [14-17]. However, the hemagglutinin protein of the Influ-

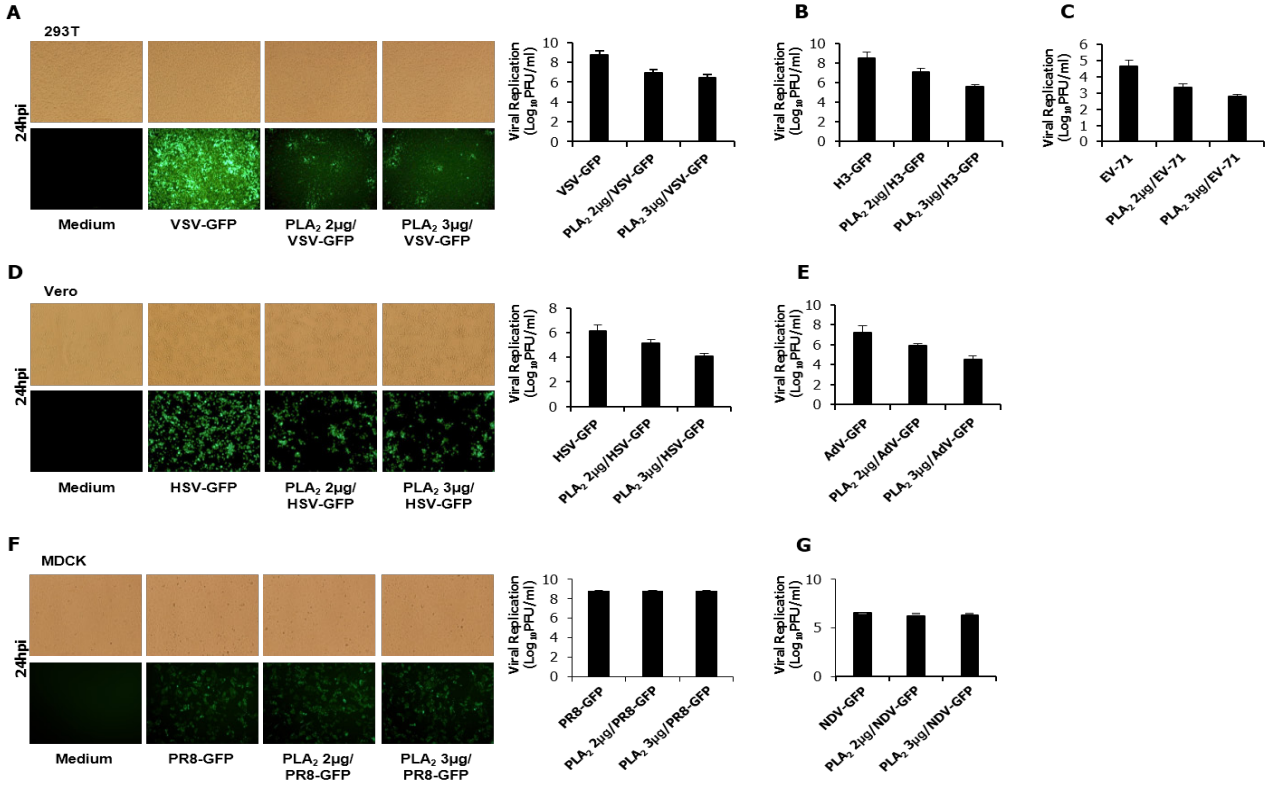

Fig. 1. *In vitro* virus attachment assay. (A) HEK293T, or (B) and (C) HeLa cells were treated with $bvPLA_2$ (2.0 and 3.0 ug/mL) for 1 hr and infected with VSV-GFP or H3-GFP or EV-71. (D) Vero, or (E) HEK293T cells were treated with $bvPLA_2$ (2.0 and 3.0 ug/mL) for 1 hr and infected with HSV-GFP or AdV-GFP. (F) MDCK, or (G) Vero cells were treated with $bvPLA_2$ (2.0 and 3.0 ug/mL) for 1 hr and infected with PR8-GFP or NDV-GFP. (A-G) At 24hpi, cells were harvested for measuring GFP absorbance and virus replication.

enza virus and the hemagglutinin-neuraminidase protein of the Newcastle disease virus (NDV) interact with sialic acid as a cellular receptor to form virus-to-cell binding complexes [18, 19]. On the basis of these previous reports and our results, we assumed that cellular HS might be a target of bvPLA$_2$.

First, we checked whether HS which acts as a cellular receptor could affect HSV-to-cell interaction. As shown in Fig. 2A and 2B, HS has an inhibitory effect on the attachment of HSV to the cell surface dose dependently, which was inconsistent with bvPLA$_2$ treatment. These data suggest that treated HS acts competitively with cellular HS to interact with virions. In order to evaluate whether bvPLA$_2$ binds to HS on the cell surface, we performed an attachment assay of HSV through co-incubation with bvPLA$_2$ and HS (Fig. 2C).

Dose-dependent bvPLA$_2$ and HSV-GFP were incubated in the cells with HS (0.5ug) for 1 h at 4ºC. After washing, the cells were transferred to 37ºC, and GFP absorbance was measured at 24 hpi. As shown in Fig. 2C, bvPLA$_2$ or HS inhibited the viral replication. However, in case that bvPLA$_2$ was added to the cells with HS, viral replication did not increase dramatically compared with that in cells treated only with HS. These results suggest that bvPLA$_2$ inhibits viral replication not by blocking HS but by blocking some other receptors on the cell surface.

The coxsackie virus and adenovirus receptor (CAR) is a receptor for the group B Coxsackie virus and subgroup C Adenovirus [20]. Therefore, we investigated whether bvPLA$_2$ binds to CAR by a fluorescence-activated cell sorting (FACS) analysis. Vero cells were treated with bvPLA$_2$ in a dose-dependent manner for 1 h and fixed with paraformaldehyde (PFA). Then, the cells were incubated with an FITC-conjugated CAR antibody (Thermo-Fisher, Korea) and subjected to FACS analysis. As shown in Fig. 2D, no change was observed in bvPLA$_2$-treated cells compared with that in mock-treated cells.

In this study, although we could not demonstrate the exact mechanism how bvPLA$_2$ blocks the cellular receptors involved in viral attachment, we found that it inhibits the replication of some viruses by blocking their attachment to the cell surface. Further studies are warranted to determine the specific target receptors of bvPLA$_2$ that block the attachment of viruses and the reason why bvPLA$_2$ did not inhibit the replication of PR8 and NDV. However, our observations suggest the possibility that bvPLA$_2$ could be used as a prophylactic antiviral reagent against some RNA and DNA viruses.

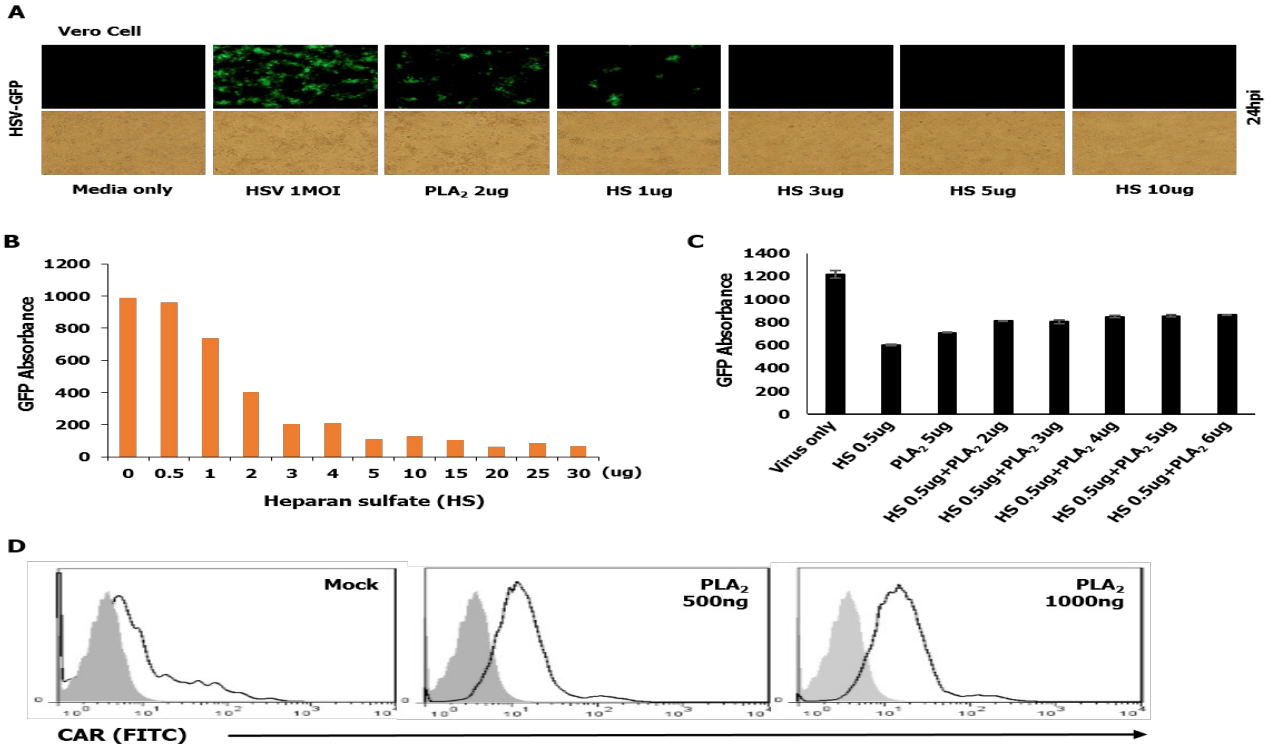

Fig. 2. Effect of bvPLA$_2$ on heparan sulfate or CAR. (A) Vero cells were incubated with bvPLA$_2$ or heparan sulfate (HS) or both as indicated dose before 1 hr of HSV-GFP infection. At 24 hpi, cells were used for fluorescence images and (B) harvested for measuring GFP absorbance. (C) HS was incubated with bvPLA$_2$ in a dose-dependent manner prior to be treated. Then, the cells were treated with HS mixed with bvPLA$_2$ for 1 hr and infected with HSV-GFP, followed by measuring GFP absorbance. (D) Vero cells were treated with bvPLA$_2$ in a dose-dependent manner for 1 hr and fixed with PFA. Cells were incubated with FITC-conjugated CAR antibody, followed by FACS analysis.

Acknowledgements

The authors thank Dr. J. U. Jung of the University of Southern California, USA for providing Green Fluorescence Protein (GFP)-fused viruses. This work was supported by the research fund of Chungnam National University in 2015.

ORCID

Jong-Soo Lee, http://orcid.org/0000-0001-5119-0711

References

1. Lee JD, Park HJ, Chae Y, Lim S. An overview of bee venom acupuncture in the treatment of arthritis. Evid Based Complement Alternat Med 2005;2:79–84.
2. Lee JY, Kang SS, Kim JH, Bae CS, Choi SH. Inhibitory effect of whole bee venom in adjuvant-induced arthritis. In Vivo 2005;19:801–805.
3. Liu XD, Zhang JL, Zheng HG, Liu FY, Chen Y. Clinical randomized study of bee-sting therapy for rheumatoid arthritis. Zhen Ci Yan Jiu 2008;33:197–200.
4. Habermann E. Bee and wasp venoms. Science 1972; 177:314-322.
5. Sobotka AK, Franklin RM, Adkinson NF Jr, Valentine M, Baer H, Lichtenstein LM. Allergy to insect stings. II. Phospholipase A: The major allergen in honeybee venom. J Allergy Clin Immunol 1976;57:29–40.
6. Von Allmen CE, Schmitz N, Bauer M, Hinton HJ, Kurrer MO, Buser RB, Gwerder M, Muntwiler S, Sparwasser T, Beerli RR, Bachmann MF. Secretory phospholipase A2-IID is an effector molecule of CD4+CD25+ regulatory T cells. Proc Natl Acad Sci 2009;106:11673–11678.
7. Boilard E, Lai Y, Larabee K, Balestrieri B, Ghomashchi F, Fujioka D, Gobezie R, Coblyn JS, Weinblatt ME, Massarotti EM, Thornhill TS, Divangahi M, Remold H, Lambeau G, Gelb MH, Arm JP, Lee DM. A novel anti-inflammatory role for secretory phospholipase A2 in immune complex-mediated arthritis. EMBO Mol Med 2010;2:172–187.
8. Nevalainen TJ, Graham GG, Scott KF. Antibacterial actions of secreted phospholipases A2.Review. Biochim Biophys Acta 2008;1781:1-9.
9. Lee G, Bae H. Bee Venom Phospholipase A2: Yesterday's Enemy Becomes Today's Friend. Toxins 2016; 8:48.
10. Ye M, Chung HS, Lee C, Yoon MS, Yu AR, Kim JS, Hwang DS, Shim I, Bae H. Neuroprotective effects of bee venom phospholipase A2 in the 3xTg AD mouse model of Alzheimer's disease. J Neuroinflammation 2016;13:10.
11. Palm NW, Rosenstein RK, Yu S, Schenten DD, Florsheim E, Medzhitov R. Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. Immunity 2013;39:976-985.
12. Muller VD, Soares RO, dos Santos-Junior NN Jr, Trabuco AC, Cintra AC, Figueiredo LT, Caliri A, Sampaio SV, Aquino VH. Phospholipase A2 Isolated from the Venom of Crotalus durissus terrificus Inactivates Dengue virus and Other Enveloped Viruses by Disrupting the Viral Envelope. PLoS One 2014;9:e112351.
13. Bernfield M, Gotte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem1999;68:729-777.
14. WuDunn D, Spear PG. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. J Virol 1989;63:52–58.
15. Dechecchi MC Tamanini A, Bonizzato A, Cabrini G. Heparan sulfate glycosaminoglycans are involved in adenovirus type 5 and 2-host cell interactions. Virology 2000;268:382–390.
16. Jackson T, Ellard FM, Ghazaleh RA, Brookes SM, Blakemore WE, Corteyn AH, Stuart DI, Newman JW, King AM. Efficient infection of cells in culture by type O foot-mouth disease virus requires binding to cell surface heparan sulfate. J Virol 1996;70:5282–5287.
17. Hallak LK, Kwilas SA, Peeples ME. Interaction between respiratory syncytial virus and glycosaminoglycans, including heparan sulfate. Methods Mol Biol 2007;379:15-34.
18. Weis W, Brown JH, Cusack S, Paulson JC, Skehel JJ, Wiley DC. Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. Nature 1988;333:426-431.
19. Huang Z, Panda A, Elankumaran S, Govindarajan D, Rockemann DD, Samal SK. The Hemagglutinin-Neuraminidase Protein of Newcastle Disease Virus Determines Tropism and Virulence. J Virol 2004;78:4176-4184.
20. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 1997;275:1320-1323.