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ALTERNATIVE MEDICINE: PLA_2 EFFECT FROM *DABOIA SP*. VENOM IN CANCER THERAPY

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ABSTRACT

PLA_2 is a major enzymatic of *Daboia* species venom which has broad pharmaceutical effects. There are many reports on PLA_2 against various cancer cells from a variety of animal venoms. Nevertheless, there was a few publishes from *Daboia* species venom on cancer. Here, we accumulated the reports on hydrolysis function of PLA_2s from snake venom on cancer cells especially from *Daboia siamensis* and *Daboia russelii russelii*. In addition, it also showed the effects from less to mild on normal cells. Overall, these findings have proved that PLA_2 property could be potent various anticancer agents. Further studies are required to elucidate the mechanism of this enzyme which induced anticancer activities. However, the efforts to better integrate research and innovation cooperation especially researchers in the housing area of this snake should look forward to the future.

KEYWORDS: alternative medicine, phospholipase A_2, *Daboia sp*. venom, cancer therapy.

INTRODUCTION

Daboia species (Russell's viper) is found in wild geographical area throughout Asia. It has been classified into five subspecies as *Daboia siamensis* (Thailand, Burma, Cambodia and Southern China), *Daboia russelii pulchella* (Sri Lanka), *Daboia russelii russelii* (India), *Daboia russelii formosensis* (Taiwan) and *Daboia russelii limitis* (Java, Komodo, Flores and Lomben).[1,2,3]

Russell's viper venom is a mixture protein, peptide both enzymatic and non-enzymatic belonging on heamatotoxic group. Although the difference of habitat area has influence on various bioactive compound of *Daboia* sp. venom, their function has still caused severe coagulophaty, renal failure and pituitary haemorrhage in patients especially in Myanmar.[4] The amounts of agents play in various important pharmaceutical properties, one of them being PLA_2.

PLA_2 enzyme is one of the main components found in this snake venom. The enzyme hydrolyzes glycerolphospholipids at the *sn*-2 position of the glycerol backbone releasing lysophospholipids and fatty acids.[2,5] PLA_2 has wildly pharmacological effects such as pre/post-synaptic neurotoxicity, myotoxicity, cardiotoxicity, pro/antiplatelet activity, edema inducing activity, hemolysis.[6] These activities may be modulated by the specific receptors located on the target cells.[7] Interestingly, it was not toxic to normal cell which was the advantage point of PLA_2 enzyme.[8,9] Although, this enzyme has potent in many cancer cells but there was a few reports from PLA_2 of *Daboia siamensis* venom. Therefore, I would like to introduce the anticancer effects of enzyme on different cancer cell lines.

Mechanism of PLA_2 on the cells

PLA_2s are enzymes which catalyze the hydrolysis of the phospholipid leading to release lysophospholipids and fatty acids.[10] Their products play either themselves as bioactive or serve as a precursor of important eicosanoids which is the major biological activity.[8] The polar products of the hydrolysis enzyme, such as palmitic acid and 1-palmitoyl-2-hydroxy-sn-glygero-3-phosphocholine or oleic acid and 1-oleoyl-2-hydroxy-sn-glycero3-phosphocholine, accumulate in the membrane merging the film surface. The surface free energy of membrane is changed and leads to change of the membrane structure and function which may cause cell damage.[5] The PLA_2s snake venom catalyze phospholipids and contain aspartic acid residue at position 49 (D49) which shown as the calcium-binding loop. However, some snake PLA_2s, which have aspartic acids residue at position 49, are substituted by lysine, serine, asparagines or arginine (K49, S49, N49 and R49) cause unable binding to Ca2+ leading to loss of the lipolytic activities. Thus, they display myotocxic and cytotoxic effects via a Ca^{2+}-independent which was a non-hydrolytic mechanism.[10] Lysine 49 phospholipase A_2 (Lys49-PLA_2) has the range of biological activities as myotoxic, cytotoxic and edema inducing effects via non-hydrolytic mechanism.[11] However, some PLA_2s could not hydrolyze phospholipids membrane of HT-29 (colon adenocarcinoma) cell, which contain an abundance of phosphatidylcholine (PC) within 24 hr of incubation.[12]

The diverse of membrane surface properties such as membrane fluidity, surface charge and structural charge cause different hydrolysis in membrane phospholipids. The ratio of saturated/unsaturated fatty acids affords PLA_2 effects. It also shows a preference for short chain fatty acids in fluid phase of membrane. In addition, some PLA_2s also present the specific surface topology region which interacts with the membrane surface of target cells. MVL-PLA_2 affects on tumor cells independent of the catalytic activity. It acts through α5β1 and αv integrins to prevent adhesion and migration of the cells.[13] On the other hand, PLA_2 molecule, which has a high anti-coagulant activity, would be located in a positive charge region of primary structure. PLA_2 with moderate or low anti-coagulant activities has specific binding located in negative charge.[7] Moreover, targeted-membrane of NnaPLA_2 and notexin relate to phosphotidylserine (PS) externalization of cell surface which cause the susceptibility of the outer or inner leaflets of phospholipids in apoptotic cells.[14]

PLA_2s from snake venom in cancer cells

The effects of PLA_2s and many bioactive compounds have screened with cytotoxic activity, inhibition of migration, invasion and adhesion methods on the cancer cells. They are a simple, rapid and high through put of results. Many PLA_2 from various snake venoms were isolated for cancer cell investigation. In 1988, Faure and Bon isolated Crotoxin, cytotoxic PLA_2 from *Crotalus durissus terrificus* venom.[15] Then, Rudd and his team (1994) found it showed cytotoxic activity against various murine and human tumor cells.[16] In 2009, Zouari-Kessntini and his colleague purified 2 non-toxic PLA_2 (CC-PLA-1 and CC-PLA-2) from *Cerastes cerastes* venom (Tunisian snake). Both enzymes inhibited adhesion of IGR39 melanoma and HT1080 fibrosarcoma cells. Moreover, it has potent migration inhibition of HT1080 cell. They claimed that it was the first report in migration inhibition of PLA_2.[17] In addition, MVL-PLA2 inhibits tumor cells independent catalytic enzyme through α5β1 and αv integrins to prevent adhesion and migration of the cells.[13] Diverse habitat provides the divergent activities, thus the initially study of the enzyme has been screening on all simple methods.

The program of cell death, apoptosis and necrosis are also detected for anticancer agents especially apoptosis. Researcher has been seeking the natural bioactive which is the selective killer to the harmful cells and gentle for the normal cells. Mediators relate to cell death, have been investigated in the level of cellular, protein, gene expression and cell death pathways. Sharkawi (2015) revealed that the PLA_2 from *Naja haje* venom combined with Melittin (Bee) venom gave a synergistic effect on the expression of p53 and BAX in liver and breast cells. Moreover, antitumor activity may be due to indirect phenomenon of inflammatory responses.[18] The results have been confirmed in various animals instead of human. Chen and his colleagues (2014) revealed that phospholipase A_2 of *Naja naja atra*, Taiwan cobra, suppressed ADAM17 maturation via ERK inactivation. This led to reduce the secreted TNF-α production which related to anti-inflammatory mechanism in human leukemia U937 cells.[19]

PLA_2 from Russell's viper venom in cancer cells

There are a few articles reported that PLA_2 of *Daboia sp*. venom could act on cancer cells by inducing apoptosis, cytotoxic activity, antiproliferative activity, and inhibition of cell migration.

Daboia siamensis (*Daboia russeli siamensis*) is a venomous viper species that is distributed throughout Southeast Asia, including Thailand. It is responsible for frequent bites causing morbidity and mortality among rural areas. Several toxins had been isolated from *D. siamensis*, including such as serine protease, phospholipase A_2, and metalloproteinase. Many reports exhibited multi-biological activities of this snake venom including anti-proliferative ones. Unfortunately, the rarely data published and have not received an appropriate attention. There was only a few PLA_2 from this venom which mentioned as cancer inhibitor and main mode of study was limited in *vitro*. Khunsap (2011) purified Drs-PLA_2 from *Daboia russeli siamensis* venom (Thai Russell's viper). Drs-PLA_2, a basic PLA_2 displayed the indirect hemolytic, anticoagulant activity, cytotoxic activity, inhibited migration and also inhibited the colonization of B16F10 cell in lung of mice.[20] This work showed the multifunctional of PLA_2 of this snake which had great functions both laboratory and animal. It seems to be an initial of PLA_2 report which focused on cancer cells inhibition, as a basic research and partially of animal experiments. After that, the enzyme showed inhibited the expression of Notch I, Notch II, Notch III and BRAF V600E genes on SK-MEL-28 cells.[9] This work was a little progression of PLA_2 effect which indicated that it may specific to extracellular receptor on cell membrane. Therefore, a lot of investigation of this enzyme should be consideration for further following in issues. 1). Determination of PLA_2 cytotoxicity should always detect in normal cell both *in vitro* and *in vivo*. 2). Does PLA_2 from snake venom interfere or participate in the proliferation of normally diving cells and also along with tumor cells? 3). The anti-adhesive PLA_2 may affect the integrity of the endothelium of the blood vessels which cause foster hemorrhage. 4). The structural features of snake PLA_2 should be consideration because it may affect the function of mammalian PLA_2.[21] However, so far require systematization and clearly focusing for further targeted works.

Daboia russelli russelli (Indian Russell's viper) is one of the most dangerous snakes in India. It causes life-threatening snake bite prevalent in India which is responsible for thousands of death each year. The major cause behind mortality due to *Daboia russelli russelli* bite is the complexcity of its venom. The venom in general contains a number of phaspholipase A_2 with different isoforms, coagulation factor V and X activating

protease, hyaluronidase, haemorrhagins, and several other constituents. PLA_2, a major component of russell's viper venom, exerts a variety of toxic effects including neurotoxicity, myotoxicity, cardiotoxicity, necrotic, haemolytic, anticoagulant, edema inducing activities and membrane damage.[22] In India, the regional (eastern, western, southern, and northern) variations in venom composition are responsible for their differences in pharmacological properties of Indian Russell's viper venom. Antivenom seemed relatively ineffective in preventing the viper venom induced renal failure, haemorrhage, tissue necrosis, and often produces serum reactions. Remarkably, it has been reported that antiserum able to neutralize the lethal toxicity of western, northern and southern regional Russell's viper venom, but failed to neutralize the lethal effects of eastern regional Russell's viper venom in India. For better therapeutic efficacy of antivenoms, several works were done with *Daboia russelli russelli* venom in India to provide information to develop new therapeutics for management against Russell's viper venom. However, development of new antidote through proper characterization of these unidentified toxins is an interesting area to explore. The crude venom of *Daboia russelli russelli* from eastern India was fractioned and resolved into four major peaks. Peak IV gave maximum yield of protein, possessed lethality and was named *dr*CT-I. The molecular weight of *dr*CT-I was 7.2 kDa. It posses several range of pharmacological activities including neurotoxicity, depolarization of the membranes of excitable cells, and selective killing of certain type of tumor cells. Toxin *dr*CT-I showed anticancer activity on human leukemic cells.[23] It also showed toxicological actions on experimental animals. It was lethal in mice (LD_{50} value) but low lethal potency compared to whole venom of *Daboia russelli russelli* venom. The lethal protein *dr*CT-I showed positive PLA2 activity but MW was different from PLA2 in Viperidae venoms. Although *dr*CT-I has some common characters of PLA2 but its N-terminal amino acid sequence has homology with other snake venom cytotoxins. Many myotoxins or cytotoxins from viper venoms were reported to be phospholipase A2. Gomes (2007) reported the antiproliferative and cytotoxicity activity of *dr*CT-I , purified protein toxin from *Daboia russelli russelli* venom for the first time in agreement with a recent studies of anticarcinogenic and cytotoxic activities of crude Russell's viper venom on Ehrlich Ascites Carcinoma (EAC) tumor cells in *vivo*. The cytotoxic action of *dr*CT-I was confirmed by the MTT studies both *in vivo* and *in vitro* indicated that *dr*CT-I mediated mitochondrial dysfunction could be a key pathway for induction of cell death in cancer cells but need further studies. The observation of this work noticed that *dr*CT-I has potential modality for inhibition of progression of cancer in animal model and human cell lines and it possessed antioxidant activities. It could be speculated that *dr*CT-I have the therapeutic potential against cancer cell proliferation (both *in vivo* and *in vitro*) by inducing apoptosis but needs some detail study to prove it a future chemotherapeutic drug against cancers.[23,24] During the same year, Maity (2007) reported the purification and characterization of a low molecular weight cytotoxic phaspholipase A_2 (RVV-7) from *Daboia russelli russelli* on B16F10 melanoma cell. The results indicated that at $2.56\pm0.51\times10^{-6}$ M. of RVV-7 killed 50% melanoma cell while more than 10^{-7} M. was ineffective to the cells. Moreover, it had the potential for inhibiting tumor development in mice.[3] In 2015, Gomes and co-author (2015) established the anticancer activity of purified fraction of Indian Russell's viper venom in cell line and animal model. They found that *dr*CT-II toxin with 6.6 KDa showed significant cytotoxic activity on leukemic cell lines and EAC bearing mice. Both *dr*CT-I and *dr*CT-II were cationic protein. There was difference in their first 20 N-terminal amino acid sequence (*dr*CT-I: LKCNKLVPLFKTCPAGKNL; *dr*CT-II: LQXNKLVPIASKTXPPGKNL). The results showed that the cytotoxicity of *dr*CT-II was less than the cytotoxicity of *dr*CT-I on human leukemic cells.[25] Fragmentation of DNA due to apoptogenic trigger requires activation of nucleases such as caspases. Caspase activation presents a late and common stage to all cells undergoing apoptosis. Caspase 3 shares both caspase 9 and caspase 8 mediated pathway of apoptogenic signaling. The *dr*CT-II was able to upregulate caspase 3 and caspase 9 in leukemic cells. Extrinsic and intrinsic apoptotic pathways can be activated separately by the activation of caspases. Caspase 9 is the initiator caspase for apoptosis in the intrinsic pathway, which then activated caspase 3. Caspase 3 cleaves and activates several caspases resulting in apoptosis. It could be concluded that *dr*CT-II from *Daboia russelli russelli* venom would be a novel pro-apoptotic agent that induced cancer cell killing through p53 and caspase pathway.[23,25]

CONCLUSION

Natural products from plants and animals play one of the major novel drug designs and developments. Recently, there are a lot of drug for treatment of diseases such as cardiovascular disease, diabetes, hypertension, multiple sclerosis and pain.[26] There are many PLA_2s from animals exhibit wildly spectrum of pharmacological effects.[18,27] PLA_2 has also been shown the potent various antitumoral. Unfortunately, there is rarely report to anticancer function by PLA_2 of *Daboia* sp. venom. Apart from PLA_2 enzyme, there are Methalloproteinase, L-amino oxidase, small peptides and the other protein also reported in broad pharmaceutical properties. The disadvantage of drug chemotherapy, include rapid cell damage, especially normal cells. The effects of PLA_2, which attack to cell membrane, lead to the study of snake venom as anticancer. *Daboia siamensis* venom and PLA_2 have just only killed the tumor cells with no harm to normal cells. This is the great advantage of them. Moreover, low MW PLA_2 toxins, *dr*CT-I and *dr*CT-II, could be the novel therapeutic potential agents against cancer cell proliferation by inducing apoptosis. However, many interesting of PLA_2 should keep seeking for further appropriate properties.

REFERENCES

1. Jigni P, Sarkar A, Chakrabarty D, Mondal S. Daboialectin, a C-type lectin from Russell's viper venom induces cytoskeletal damage and apoptosis in human lung cancer cells inÂ vitro. Food and agriculture organization of the United Nations, 2017; Available from: http://www.nal.usda.gov/
2. Saikia D, Thakur R, Mukherjee AK. An acidic phospholipase A_2 (RVVA-PLA_2-I) purified from *Daboia russelli* venom exerts its anticoagulant activity by enzymatic hydrolysis of plasma phospholipids and by non-enzymatic inhibition of factor Xa in a phospholipids/Ca^{2+} independent manner. Toxicon, 2011; 57: 841-850.
3. Maity G, Mandal S, Chatterjee A, Bhattachaya D. Purification and characterization of a low molecular weight multifunctional cytotoxic phospholipase A_2 from Russell's viper venom. Journal of chromatography B, 2007; 845: 232-243.
4. Than Yee K, Tongsima S, Vasieva O, Ngamphiw C, Wilantho A, Wilkinson MC, Somparn P, Pisitkun T, Rojnuckarin P. Analysis of snake venom methalloproteinases from Myanmar Russell's viper transcriptome. Toxicon, 2018; 146: 31-41.
5. Jurak M, Szcześ A, Chibowski E. Physicochemical properties of phospholipid model membranes hydrolyzed by phospholipase A_2 (PLA_2) in the presence of cholesterol at different temperatures. Applied surface science, 2013; 266: 426-432.
6. Kumar JR, Basavarajappa B, Vishwanath BS, Gowda V. Biochemical and pharmacological characterization of three toxic phospholipase A_2s from *Daboia siamensis* snake venom. Comparative biochemistry and physiology, psrt C, 2015; 168: 28-38.
7. Bonfim VL, Ponce-Soto LA, Martins de Souza D, Souza GHMF, Baldasso PA, Eberlin MN, Marangoni S. Structural and functional characterization of myotoxin, Cr-IV 1, a phospholipase A_2 D49 from venom of snake *calloselasma rhodostoma*. Biological, 2008; 36: 168-176.
8. Calderon LA, Sobrinho JC, Zaqueo K, de MouraAA, Grabner AN, Mazzi MV, Marcussi S, Nomizo A, Fernandes CFC, Zuliani JP, Carvalho BMA, da Silva SL, Stábeli RG, Soares AM. Antitumoral activity of snake venom proteins: New trends in cancer therapy. BioMed Research International, 2014; ID203639, http://dx.doi.org/101155/2014/203639.
9. Khunsap S, Khow O, Buranapraditkun S, Suntrarachun S, Puthong S, Boonchang S. Anticancer properties of phospholipase A_2 from *Daboia siamensis* venom on human skin melanoma cells. Journal of venomous animals and toxins including tropical disease, 2016; DOI:10.1186/s40409-016-0061-z, 2016.
10. Ghazaryan NA, Ghulikyan L, Kishmiryan A, Andreeva TV, Utkin YN, Tsetlin VI, Lomonte B, Ayvazyan NM. Phospholipase a_2 from *Viperidae* snakes: Differences in membranotropic activity between enzymatically active toxin and its inactive isoforms. Biochimica et Biophysica Acta, 2015; 1848: 463-468.
11. Chioata L, Ward R.J. Mapping structural determinants of biological activities in snake venom phospholipase A_2 by sequence analysis and site directed mutagenesis. Toxicon, 2003; 42: 869-883.
12. Saikia D, Bordoloi NK, Chattopadhyay P, Choklingam S, Ghosh SS, Mukherjee AK. Differential mode of attack on membrane phospholipids by and acidic phospholipase A_2 (RVVA-PLA_2-I) from *Daboia russelli* venom. Biochimica et Biophysica Acta, 2012; 3149-3157.
13. Bazaa A, Luis J, Srairi-Abid N, Kallech-Ziri O, Kessentini-Zouari R, Defilles C, Lissitzky J-C, Ayeb M.E, Marrakchi N. MVL-PLA2, a phospholipase A2 from *Macrovipera lebetina transmediterranea* venom, inhibit tumor cells adhesion and migration. Matrix Biology, 2009; 188-193.
14. Chiou Y-L, Lin S-R, Hu W-P, Change L-S. Modulated mechanism of phosphatidylserine on the catalytic activity of *Naja naja atra* phospholipase A_2 and *Notechis acutatus scutatus notexin*. Toxicon, 2014; 113-122.
15. Faure G, Bon C. Crotoxin, a phospholipase A_2 neurotoxin from the South American rattlesnake Crotalus durissus terrificus: purification of several isoforms and comparison of their molecular structure and of their biological activities. 1988; 27(2): 730-738. DOI: 10.1021/ bi00402a036.
16. Rudd CJ, Viskatis LJ, Vidal JC, Etcheverry MA. In vitro compariirison of cytotoxic effects of crotoxin against three human tumors and a normal human epidermal keratinocyte cell line. Invest new drugs, 1994; 12 (3): 183-4.
17. Zouari-Kessentini R, Luis J, Karray A, Kallech-Ziri O, Srairi-Abid N, Bazaa A, Loret E, Bezzine S, Ayeb ME, Marrakchi N. Two purified and characterzed phospholipase A_2 from *Cerastes cerastes* venom that inhibit cancerous cell adhesion and migration. Toxicon, 2009; 444-453.
18. Sharkawi FZE, Saleh SS, Sayed AFME. Potential anti cancer activity of snake venom, Bee venom and their components in liver and breast carcinoma. International journal of pharmaceutical sciences and research, 2015; 3224-3235.
19. Chen Y-J, Lin H-C, Chen K-C, Lin S-R, Cheng T-L, Chang L-S. Taiwan cobra phospholipase A_2 suppresses ERK-mediated ADAM17 maturation, thus reducing secreted TNF-α production in human leukemia U937 cells. Toxicon, 2014; 79-88.
20. Khunsap S, Pakmanee N, Khow O, Chanhome L, Sitprija V, Suntravat M, Lucena SE, Perez JC, Sánchez E. Purification of a phospholipase A_2 from *Daboia russelii siamensis* venom with anticancer effects. J Venom Res, 2011; 42-51.
21. Osipov AV, Utkin YN. Antiproliferation effects of snake venom phospholipase A_2 and their

perspectives for cancer treatment. Toxins and Drug discovery, 2015; 1-15. DOI: 10.1007/ 978-94-007-6726-3_13-1
22. Bhattacharjee P, Bhattacharyya D. Therapeutic use of snake venom components: A voyage from ancient to modern India. Mini Reviews in Organic Chemistry, 2014; 11: 45-54.
23. Gomes A, Choudhury SR, Saha A, Mishra R, Giri B, Biswas AK, Debnath A, Gomes A. A heat stable protein toxin (drCT-1) from the Indian Viper (*Daboia russelli russelli*) venom having antiproliferative, cytotoxic and apoptotic activities. Toxicon, 2007; 49: 46-56.
24. Ahluwalai S, Sawant MG, Shah N, Chowdhary A. Experimental evaluation of antitumor effect of Russell's viper venom on breast cancer cell line – MDA – MB – 231. 2015. Available from: http://ijppr.humanjournals .com/
25. Gomes A, Biswas AK, Bhowmik T, Saha PP, Gomes A. Russell's viper venom purified toxin Drct-II the cell proliferation and induces G1 cell cycle arrest in human leukemic cancer cells. Transl Med, 2015; 5: 1-7.
26. Takacs Z, Nathan S, Animal venoms in medicine. In:Wexler P, editor. Encyclopedia of Toxicology. V1. 3rd ed. Elsevier Inc., Acaemic Press, 2014; 252-9.
27. Kini RM. Excitement ahead: structure, function and mechanism of snake venom phospholipase A_2 enzymes. Toxicon, 2003; 827-840.