IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 2 Ver. II (Mar. – Apr. 2018), PP 05-12
www.iosrjournals.org

Development ofNovel Phospholipase A2 Inhibitors Using Molecular and Computational Techniques

Manoj G Tyagi*#, Dinesh J Vyas, Vinita ErnestAnd Debjyoti Mukherjee
Department Of Pharmaceutical ChemistryVIT, Vellore&Department Of PharmacyMonash University School Of Medicine,Selangor, Malaysia#*
Corresponding Author:Manoj G Tyagi

Abstract:*Phospholipase A_2 Enzymesare Basically Of Three Types i.e The Cytosolic PLA_2, Secretory Phospholipase A_2And Lipoprotein Associated PLA_2. There Is Growing Interest In Developing Novel And Potent PLA_2Inhibitors For Various Therapeutic Purposes E.G Alzheimer's Disease, Allergic Conditons, Arthritis And Cancer, Cardiovascular Disorders And To Counter The Envenomation By Bee, Spider And Snake Bites. One Of The Excellent Example Is Darapladib Which Is An Effective And Potent Inhibitor Of The Lipoprotein Associated $PLA_2$and Is Used For Clinical Conditions. On The Other Hand Computational Studies Conducted On The Numerous Snake Species Having Endogenous Phospholipase A_2Inhibitors, Can Be Exploited For Therapeutic Purposes. This Inhibitor Type Is Generally Known As Snake Blood Phospholipase A_2 Inhibitors (Sbplis). Most, If Not All Sbplis Are Oligomeric Glycosylated Proteins, Although The Carbohydrate Moiety May Not Be Important For PLA_2Inhibition In Most Cases.Western Blot Analysis After Partial Purification With $SPLA_2$-IB-Affinity Column Has Confirmed The Identity Of Serum Spla2 Binding Protein As A Soluble Form Of PLA_2R ($SPLA_2$r) That Retained All Of The Extracellular Domains Of The Membrane-Bound Receptor. This Review Article Has Tried To Encompass The Recent Advances In The Development Of Novel And Potent PLA_2 Inhibitors, Both Endogenous And Synthetic In Nature.*

Key Words:*Phospholipase A_2, Inhibitors, Endogenous, Crotoxin, Lipoprotein, Molecular, Therapeutic*

Date of Submission: 05-03-2018 Date of acceptance 23-03-2018

I. Introduction:

Phospholipase A_2Enzymes Are Basically Of Three Types I.E The Cytosolic PLA_2, Secretory Phospholipase A_2And Lipoprotein Associated PLA_2. Phospholipase A2 (PLA2) Is An Enzyme That Catalyzes The Hydrolysis Of The Sn-2 Ester Bond Of Glycerophospholipids Thereby Causing The Release Of The Arachidonic Acid (1, 2). Since Its Discovery, PLA_2Has Been A Molecular Target Of Extensive Research Because Of Its Critical Involvement In Physiological And Pathological Events Such As Phospholipid Turnover And Production Of Pro-Inflammatory Lipid Mediators (3). To Date, A Number Of Mammalian Intracellular And Extracellular PLA_2s Have Been Identified And Classified Into Different Families According To Their Biochemical Features (4). Amongst Them, $Spla_{2s}$ Have Several Common Characteristics Including A Relatively Low Molecular Mass (14-18 Kda), The Presence Of 6 To 8 Disulfide Bridges, And An Absolute Catalytic Requirement For Millimolar Concentrations Of Ca^{2+} (5, 6). Recent Studies Show The Evidence For The Existence Of Circulating Phospholipase A_2Inhibitors Against Secretory PLA2s (Spla2s) In Mammals. In Mouse Serum, Detection Of Specific Binding Activities Of Group IB And X Spla2s (Spla2-IB And Spla2-X), Which Was In Contrast With The Absence Of Binding Activities In Serum Prepared From Mice Deficient In PLA2 Receptor (PLA_2R), A Type I Transmembrane Glycoprotein Related To The C-Type Animal Lectin Family. Western Blot Analysis After Partial Purification With Spla2-IB-Affinity Column Confirmed The Identity Of Serum Spla2 Binding Protein As A Soluble Form Of PLA2R ($Spla_2r$) That Retained All Of The Extracellular Domains Of The Membrane-Bound Receptor (7-8). In A Recently Published Article This Author Has Nicely Depicted The Importance Of The Lipoprotein Associated PLA_2 Also Known As The PAF Acetylhydrolase(9). There Is Growing Interest In The Development Of Both Endogenously Found And Synthetic PLA_2Inhibitors And For This Purpose Molecular And Computational Methods Are Being Exploited. This Review Article Tries To Evaluate The Recent Progress In The Development Of Novel PLA2 Inhibitors For The Purpose Of Therapeutic Usage.

CNF And Related Peptides Used For Countering The PLA_2 : The Endogenously Foundcrotalus Neutralizing Factor (CNF) – Encodes A 19-Residue Signal Peptide Characteristic Of Secreted Proteins, Followed By 181 Amino Acids In The Mature Protein, Including Sixteen Cysteines. CNF Is A Glycosylated Alpha1-Globulin With A Single N-Terminal Linked Carbohydrate Site At Asn157 (10-11). The Carbohydrate Moiety, However, Is

Not Essential For PLA_2Inhibition, Since CNF Remains Functional After Enzymatic Deglycosylation (12). The Native CNF Is A Globular-Shaped, Predominantly Tetrameric Molecule With An Average Molecular Mass Of 100 Kda In Solution. It Innately Occurs As A Mixture Of Non-Glycosylated And Glycosylated Monomers Of 22 Kda And 25 Kda, Respectively (13). The Oligomerization Of CNF Is Independent Of The Presence Of Carbohydrates, Since It Occurs Equally With Native Or Enzymatically Deglycosylated Monomers. Tyrosine Residues At The Interface Of The Monomers Composing CNF May Contribute To The Oligomerization Process, According To A Proposed Theoretical Structural Model Constructed For The Inhibitor Available With DOI:10.5452/Ma-Avb44 At Modelarchive Database. The U Monomer Of The Crystallographic Structure Of Urokinase Plasminogen Activator From Homo Sapiens (PDB ID: 2FD6) Was Used As The Template *Ab Initio* (14). Besides Inhibiting Lethal And PLA2 Actions Of C. D. Terrificus Venom, CNF Is Also Able To Inhibit The Lethal Activity Of Heterologous Viperid Venoms, Such As Those From Bothropsalternatus, B. Atrox, B. Jararaca. B. Jararacussu, B. Moojeni, B. Neuwiedi And Lachesis Muta, But Not That Of The Elapid Micrurus Frontalis (15).The Natural Target Of CNF In Homologous Venom Is Crotoxin, A Heterodimeric B-Neurotoxin Formed By An Enzymatically Inactive Subunit (Crotoxin A Or CA) And A PLA_2 Counterpart (Crotoxin B Or CB). CA And CB Are Non-Covalently Bonded In The Crotoxin Complex (CA/CB). CNF Is Able To Displace CA In The Native Crotoxin In Vitro To Form A Non-Toxic CNF/CB Complex, Most Likely At A 1:1 Molar Ratio (16). In The Presence Of CNF, The Newly Formed CNF/CB Complex No Longer Interacts With The Target Acceptor Of Crotoxin On Rat Brain Synaptosomes To Deliver CB To Cause Its Neurotoxic Effect.

Lp-PLA_2 Inhibitors:PAF Acetylhydrolase Or LP-PLA2 Is An Important Enzyme Under Intensive Scrutiny.An Alternative Permeability-Inducing Agent In Diabetic Retina Could Be Lysophosphatidylcholine (LPC), Which Is Increased In Plasma Of Diabetic Patients (17) And Has Demonstrated Permeability Enhancing Activity In Cultured Non-Neural Endothelial Cells (Ecs). The Principal Enzyme Responsible For The Production Of LPC Is A Calcium-Independent Phospholipase A_2Called Lipoprotein-Associated Phospholipase A_2(Lp-PLA_2) Which Is Also Known Astype VIIA PLA2(18). Darapladib, A Specific Inhibitor Of Lp-PLA_2, Has Been Shown To Reduce Atherosclerosis In Both Diabetic/Hypercholesterolemic Pigs (19-20) And Apoe-Deficient Mice (21). In Diabetic/Hypercholesterolemic Pigs, Darapladib Protected Against Blood–Brain Barrier (BBB) Dysfunction And Vascular Permeability. Darapladib Has Been Studied In Nearly 16,000 Patients With Coronary Heart Disease, And Approximately Onethird Of This Study Population Had Diabetes Mellitus (22). In A Further Study, A 3-Month Daily Treatment With 160 Mg Of Darapladib Orally Showed Reduction Of DME And An Improvement In Visual Acuity In Patients (23).Another Lp-PLA2 Inhibitor And Congener Developed By Glaxo Smith Kline i.e GSK2647544 Is A Potent And Specific Inhibitor Of Lipoprotein-Associated Phospholipase A_2 (Lp-PLA_2), Which Was In Development As A Potential Treatment For Alzheimer's Disease (AD). In Order To Refine Therapeutic Dose Predictions And Confirm Brain Penetration, A Radiolabelled Form Of The Inhibitor, [^{18}F]GSK2647544, Was Developed For Use In A Positron Emission Tomography (PET) Biodistribution Study. The Study Provides Evidence That GSK2647544 Is Able To Cross The Blood Brain Barrier In Healthy Male Subjects Leading To A Measurable Brain Exposure. The Administered Doses Of GSK2647544 Were Well Tolerated. Exploratory Modelling Studies Suggested That A Twice-Daily Dose Of 102 mg, At Steady State, Would Induce ~80 % Trough Inhibition Of Brain Lp-PLA_2 Activity. Thus New Congeners Which Effectively Inhibit The Lp-PLA2 Are Being Investigated.

Computational Studies On Novel PLA2 Inhibitors: There Are Many Reports Where Computational Molecular Modeling Methods Have Been UsedFor Characterizing Some Functional Aspects Of PLA_2s, Or The Development Of PLA2 Inhibitors That Contribute To The Attenuation Or Annihilation Of Snake Venom Toxicity. These Applications Use The X-Ray Crystallographic 3D Structural Information Generated In The Last Few Decades, And Methods Such As Molecular Dynamics (MD) Simulations And Docking. Structural Architecture Of Snake Venom PLA2s Is Divided Into Classes I And II, Based On Their Amino Acid Sequence And Disulfide Bonding Pattern (24). However, They Have A Conserved Structure Which Contains An N-Terminal A-Helix (H1), A Ca^{2+}Binding Loop, Two Antiparallel A-Helices (H2 And H3), A Two-Stranded Antiparallel Sheet (B-Wing), And A Long C-Terminal Loop. In General, Folding Is Stabilized By Seven Disulfide Bonds With Different Types Of Pattern In Classes I And II. Some PLA2s Undergoaggregation In A Concentration-Dependent Manner. Crystal Structures Available For Several PLA2s Confirm That They Can Form Associations In Dimer, And More Units With Physiological Implications. The Majority Of Molecular Modeling Applications In Literature For Studying PLA2s Are Oriented To Rational Design Of Novel Inhibitors For The Treatment Of Different *Viperidae*snakebites. Some Examples Are Cited Here Which Are Described: Most Examples Have Been Applied To PLA2 Of *Daboiarusselii*. Recently, Nargotra et al (25) Evaluated A Library Of Natural Products And Synthetic Molecules Through Docking Studies On *D. Russelii*PLA2 To Identify Possible Inhibitors. Their Study Lead To *In Silico* Identification Of Several Molecules As PLA2 Inhibitors, With Most Of Them Belonging To Phenolic And Substituted Benzaldehydiccompounds.It Is Important To Note That The Selection In This Work Was Performed By Considering Docking Energy Scores,

Which Is A Reliable Criterion, According To Literature. The Same Authors Proposed The Docking Poses Inside PLA_2Of *D.Russelii*for Synthetic Phenolic Compounds Effective Against Snake Venom. They Found That Phenolic Compounds Having Hydroxyl And Methoxyl Groups In Their Benzene Ring Showed Maximum Inhibitory Potency. The Majority Of Molecular Modeling Applications In Literature For Studying PLA2s Are Oriented To Rational Design Of Novel Inhibitors For The Treatment Of Different *Viperidae*snakebites. Snake Bite Is A Serious Global Problem, Especially In Countries With Subtropical Climate Like India, Phillipines And Other South East Asian Countries. Phospholipases A2 (PLA2s) Commonly Found In Snake Venom, Are Extensively Studied Due To Their Pharmacological And Physio-Pathological Effects. Numerous Plant Species Are Used In Folk Medicine To Treat Venomous Snake Bite Without Scientific Validation. A Good Example Is The Indian Medicinal Ricenjavarawhich Is A Unique Medicinal Variety Rice In Kerala Used In Ayurveda For Many Disease Conditions Including Snake Bite Pustules. In This Published Report, Bioactive Compounds Isolated From NjavarawereScreened As Inhibitors, Against The Indian Russell 'S Viper PLA2 (PDB Id: 1TH6) Using Molecular Docking Techniques (26). Phytochemical Investigation Of Njavara Led To Isolation Of Six Compounds For The First Time Including Bioactive Phenolic Acids (Ferulic, Syringic, Vanillic And Protocatechuic Acid), B-Sitosterol, And 24 - Methylene Cycloartanylferulate.

On The Other Hand Another Form Of PLA2, Group VIA Calcium-Independent (GVIA $Ipla_2$), And Group V Secreted (GV $SPLA_2$) Enzymes Are Implicated In Many Inflammatory Diseases (27). Thus, The Development Of Potent And Selective Inhibitors For Each Of These Three Enzymes Should Lead To The Development Of Novel Pharmaceutical Agents For Different Inflammatory Conditions. GIVA $CPLA_2$ Was Cloned And Sequenced In 1991and Its Crystal Structure Was Reported In The Year 1999. This Enzyme Utilizes A Catalytic Dyad Of Ser/Asp, And It Exhibits High Specificity For Membrane Phospholipids Containing Arachidonic Acid (AA) At The *Sn*-2 Position. Thus, It Is The Main AA Provider For The Cyclooxygenase (COX) And 5-Lipoxygenase (LOX) Pathways. Therefore, This Enzyme Can Be Considered A Key Enzyme For Mediating Production Of Eicosanoids Which Are Implicated In Numerous Inflammatory Diseases (28).

A Variety Of Diverse Small Molecule Inhibitors Against PLA2 Have Also Been Reported And Their Structures Are Summarized In Some Review Articles. These Groups Have Developed Some Novel Classes Of Inhibitors Including 2-Oxoamides For GIVA $Cpla_2$amides For GV $sPLA_2$, And Fluoroketones For GVIA $IPLA_2$.They Have Now Explored Potent And Selective Inhibitors For GVIA $Ipla_2$ Using Structure-Based Design And *In Vitro* Mixed Micelle Assays.Even Though There Is No Available Crystal Structure For This Enzyme, A Robust Homology Model Was Developed Based On Hydrogen/Deuterium Exchange Mass Spectrometry (DXMS) Experimental Data And Molecular Dynamics (MD) Simulations.The 3D Structure Of GVIA
$IPLA_2$ Was Used For Molecular Docking Calculations And MD Simulations With Previously Synthesized Inhibitors In An Effort To Establish A Structure-Activity Relationship (SAR) For The Development Of Novel And Potent Inhibitors.

Common Techniques Employed In Developing A Novel PLA_2inhibitor :

Molecular Docking

Molecular Docking Can Be Done Using Several Techniques And Softwares For e.g.,The Virtual Screening And Docking Can Be Performed Using Autodockvina. Autodockvinais Used Due To Its Accuracy And Speed. Autodockvinais Utilized To Automate The Docking Process Towards The NADI Compounds. The Predicted Binding Energy (ΔG), Which Indicates The Strength Of Compounds Bind To The Receptor Is Calculated Based On Scoring Function Used In Autodockvina. The Top Ten Docking Conformations For Each Compound Is Selected Using A Python Script File. The Selection Is Based On Lowest Energy Binding. The H-Bond, And Hydrophobic Interaction Areanalysed Using Ligplotserver (29-30) And Viewed Using A Discovery Studio Visualizer (Refer Fig.2).

Inhibition Of PLA2 Activity

The Inhibitory Activity Of PLA2 Can Be Tested According To The Method Described By De Aranjo And Radvany (31). Briefly, The Substrate Consisted Of 3.5 Mm Lecithin, A Mixture Of 3 Mm NATDC, 100 Mm NACl, 10 Mm $Cacl_2$, And 0.055 Mm Red Phenol As Colorimetric Indicator, And 100 Ml H_2O. The pH Of The Reaction Mixture Was Adjusted To 7.6. 0.2 Mg Of Pg-IB Is Solubilized In 10% Acetonitrile At A 0.002 Mg/Ml Concentration. A Volume Of 2 Ml Of PLA2 Solution Is Incubated With 2 Ml Of Sample For 20 Min At Room Temperature. Then, 200 Ml Of PLA2 Substrate Was Added To The Solution. Kinetic Hydrolysis Is Performed For 5 Min, And Optical Density Is Estimated At 558 Nm. The PLA2 Inhibitory Activity Is Expressed In Inhibition Percentage And Is Calculated As Follows:

Ezyme Activity=Odzero− OD15 Min/15 Min% Inhibition=Enzyme Activity−Ve Control− Enzyme Activity sampleenzyme Activity−ve Control × 100

Immunoblotting Analyses:Immunoblotting Or Western Blotting Is An Effective Technique To Identify Novel Proteins And Their Molecular Mass.For Western Blotting Analyses, Proteins Are Transferred To A Nylon Membrane, Which Is Subsequently Blocked (1 H, Room Temperature) With Tris-Buffered Saline-Tween (TBS-T, Which Contains 20 Mm Tris-HCll, 137 Mm Nacl, Ph 7.6, And 0.1% Tween 20) Containing 1% BSA And 3% Milk Powder (32). Following Three Washes With TBS-T, The Blot Was Then Incubated (1 H, Room Temperature) With A Monoclonal Antibody (1:50 Dilution In TBS-T With 1% BSA) Raised In A Mouse Against A Synthetic Peptide Representing A 10-Amino Acid Residue Sequence (221TPLHLACQMG230) Located At The C Terminus Of Islet Cai-PLA2. The Nylon Membrane Is Washed Three Times In TBS-T And Incubated (1 H, Room Temperature) With A Goat Anti-Mouse Igg Conjugated To Horseradish Peroxidase (Boehringer Mannheim) At A 1:3000 Dilution In TBS-T Containing 1% BSA. Detection Of The Secondary Antibody Is Performed By Enhanced Chemiluminescence (Refer Fig.1).

Monoclonal Antibody Against Snake PLA2 : Monoclonal Antibodies Are Increasingly Being Used For Therapeutic Purpose In Disorders Like The Bronchial Asthma, Arthritis, Psoriasis And Cancer. The Snake Venom PLA_2s ($SvPLA_2$) Are Important Toxins And Comprise An Important Target For The Development Of New Anti-Venom Drugs. Snake And/Or Mammals Serum Are Repositories Of $Svpla_2$ Inhibitors (PLis) Due To Protective Benefits (33). Immunodetection Is An Essential Technique Commonly Employed For Protein Discovery, Quantification And Investigation. Thus, Mab Development Of Pliγ Is Technically Significant For Anti-Venom Studies.The Classical Routine Of Monoclonal Antibody Preparation Is Time Consuming And Laborious; The Resulted Mabs Are Generally Very Specific. Protein-Specific Antibodies Can Be Generated By Immunization Of Animals With Peptides, If The Peptide Is An Effective Epitope Of The Protein. Bioinformatics Prediction Followed By Concrete Experimental Validation Is Both Economical And Effective. For Epitope Prediction, Bioinformatics Software Can Reduce The Experimental Workload By 95% And Increase The Efficiency Of New Epitope Location By 10 To 20 Folds. In This Study, Dnastar Protean Program Was Used To Predict Epitopes Of Sapliγ By Comprehensively Analyzing Many Parameters Such As Hydrophilicity, Surface Accessibility, Antigenic Index, Secondary Structure And Flexibility. Finally, The ^{151}CPVLRLSNRTHEANRNDLIKVA^{172} As A Hapten And Obtained 18 Igg Mab Cell Strains. The Resulted Pliγmab Could Recognize A Broad Range Of Snake Sera Including Venomous And Non-Venomous Snake Species, Because The Epitope Peptide Is Highly Homologous Among Snake Pliγs. The Resulted Mab Is Applicable For Pliγimmunodetection Of A Wide Range Of Snake Species (34).

Natural And Synthetic Inhibitors Of PLA2:

Manoalide Was Initially Isolated From The Sponge Luffatiellavariabilis. Because Of Its Potent Anti-Inflammatory And Analgesic Effects, This Compound Is Now In Phase 1 Clinical Trial. Although This Agent Is Not Clinically Available, Manoalide Becomes A Standard Drug In Inflammation Research (35-36). The Other PLA2 Inhibitors Are Variabilin, Cacospongiolide B, Bolinaquinone, And OAS1000 (35).Bromelain And Phytochemicals Like Amenthoflavone, Asiaticoside, And Diosgenin Have Been Reported To Exhibit Inhibitory Effects Against PLA2 Activity (37-38). Bromelain, Asisticoside And Diosgenin Appear To Be Safe Compounds, As They Do Not Show Any Toxic Effects With A Lethal Dose (LD_{50}) Of Up To 750 Mg/Kg In Dogs, 50 Mg/Kg In Mice And More Than 800 Mg/Kg In Mice, Respectively. It Was Also Discovered That The Combination Of Phytochemical Compounds With Bromelain Could Enhance The Functional Properties And Thermal Stability And Increase The Shelf Life Of Pineapple Juice. The Combinations With Natural Products, Including Bromelain, Was Also Proven To Enhance The Effect Of Other Anti-Inflammatory Drugs Such As Paracetamol In The Relief Of The Knee Joint Pain (39). The Effect Of The Combination Between Bromelain And Antibiotics Was Shown To Be More Effective Compared To Antibiotics Alone In The Treatment Of Pneumonia, Bronchitis And Cellulitis (40-42).

In This Study, The Synergistic Potential Of Combinations Of Bromelain And Phytochemicals Namely, Amenthoflavone, Asiaticoside, And Diosgenin, Against PLA2 Was Quantified. The Combinations Of Bromelain-Amenthoflavone (Br-Am), Bromelain-Asiaticoside (Br-As), And Bromelain-Diosgenin (Br-Di) Were Analyzed Using A Protocol Which Is Widely Used To Determine The Synergistic And Antagonistic Effects In Combination Studies. Subsequently, Proof Of The Utility Of The Bromelian-Phytochemical Complex Were Generated By Measuring The Inhibitory Activity Against PLA2(43).Flavinoids Too Have Been Investigated For PLA2 Inhibitory Activity. The Inhibition Of $PLA_2$by Polyphenolic Flavonoids Has Been Reported In A Number Of *In Vitro* And *In Vivo* Studies. Quercetin Was Found To Be An Effective Inhibitor Of PLA_2 In Human Leukocytes. Bioflavonoids Such As Amentoflavone, Bilobetin, Morelloflavone And Ginkgetin Derived From Certain Medicinal Plants Have Been Shown To Inhibit PLA_2 As Well. Curcumin Affects Arachidonic Acid Metabolism By Blocking The Phosphorylation Of Cytosolic PLA_2, Resulting In Decreased COX-2 Expression. Since PLA_2 Is Coupled With Coxs And Loxs Depending On The Cells, PLA_2 Becomes The Molecular Target Of Polyphenols To Cause The Inhibition Of COX Or LOX Activity And Inflammation (44).

Fig.1 : Phospholipase A2 Enzyme As Detected By Western Blotting

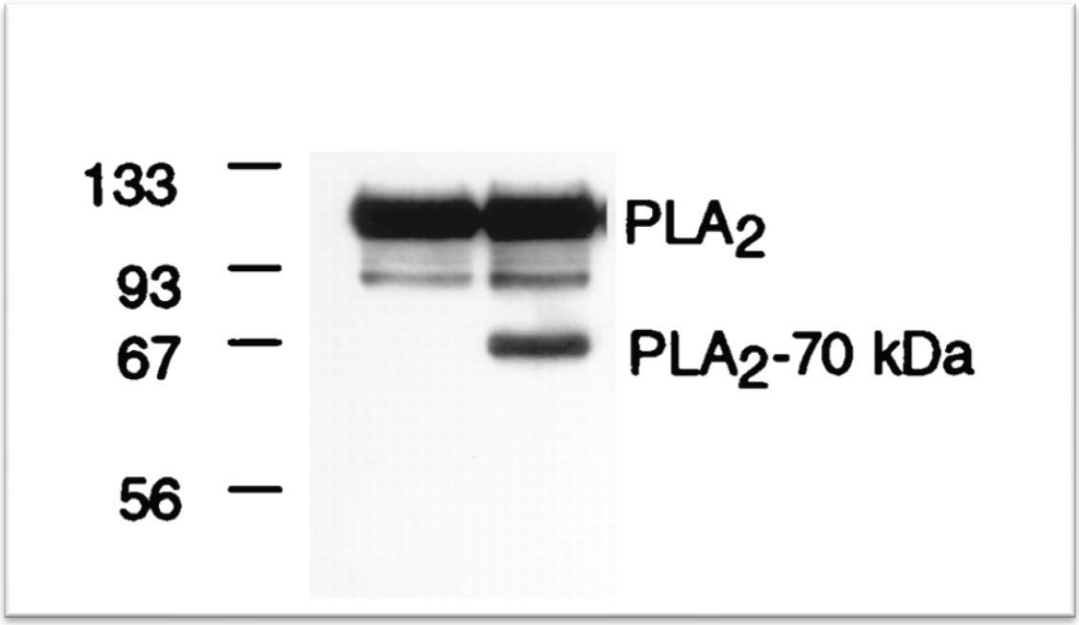
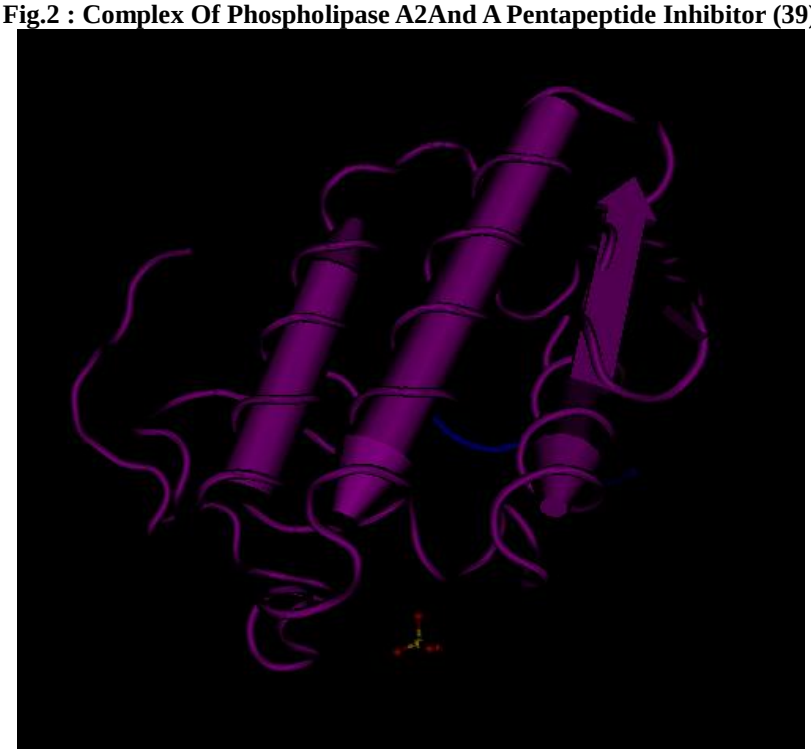


Fig.2 : Complex Of Phospholipase A2And A Pentapeptide Inhibitor (39)



II. Conclusion:

Phospholipase A_2(PLA_2) Enzymes Are A Diverse Group That Hydrolyze Membrane Phospholipids Into Arachidonic Acid And Lysophospholipids. These Lipid Mediators Play Critical Roles In The Induction, Maintenance, And Modulation Of Neuroinflammation And Oxidative Stress. Many Neurological Disorders Including Excitotoxicity; Traumatic Nerve And Brain Injury; Cerebral Ischemia; Alzheimer's Disease; Parkinson's Disease; Multiple Sclerosis; Experimental Allergic Encephalitis; Pain; Depression; Bipolar Disorder; Schizophrenia And Also Cardiovascular Disorders Are Characterized By Oxidative Stress,

Inflammatory Reactions And Alterations In Phospholipid Metabolism, Accumulation Of Lipid Peroxides, And Increased Activities Of Brain Phospholipase A_2Isoforms. Many Old And New Synthetic Inhibitors Of PLA_2, Including Fatty Acid Trifluoromethyl Ketones; Methyl Arachidonylfluorophosphonate; Bromoenol Lactone; Indole-Based Inhibitors; Pyrrolidine-Based Inhibitors; Amide Inhibitors, 2-Oxoamides; 1,3-Disubstituted Propan-2-Ones And Polyfluoroalkyl Ketones As Well As Phytochemical Based PLA_2Inhibitors Including Curcumin, *Ginkgo Biloba* And *Centellaasiatica*Extracts Have Been Discovered And Used For The Treatment Of Clinical Disorders In Cell Culture And Animal Model Systems. The Blood Of Poisonous Snakes Contain PLA_2Inhibitors Whose Structure Activity Relationship Can Be Used For Developing Potent PLA_2 Inhibitors For Treating Envenomation And Other Pathological Disorders. The Purpose Of This Review Was To Summarize Information On Selective And Potent Synthetic Inhibitors Of PLA_2As Well As Several PLA_2Inhibitors From Plants And Endogenous PLA_2Inhibitors From Marine And Snake Species For Therapeutic Purposes.

Acknowledgements: The Authors Thank Ms.Vishakha Tyagi, MSc., For Her Help Towards This Research Article.

References:

[1] Magrioti V, Kokotos G. Phospholipase A_2 Inhibitors For The Treatment Of Inflammatory Diseases: A Patent Review (2010 - Present) Expert Opinther Pat. 2013;23:333–344.

[2] Ong WY, Farooqui T, Kokotos G, Farooqui AA. Synthetic And Natural Inhibitors Of Phospholipases A_2: Their Importance For Understanding And Treatment Of Neurological Disorders. ACS Chemneurosci. 2015;6:814–831.

[3] Bucher D, Hsu YH, Mouchlis VD, Dennis EA, Mccammon JA. Insertion Of The Ca^{2+}-Independent Phospholipase A_2 Into A Phospholipid Bilayer Via Coarse-Grained And Atomistic Molecular Dynamics Simulations. Ploscomput Biol. 2013;9:E1003156.

[4] Varnavas D. Mouchlis-A, Dimitris Limnios, Maroula G. Kokotou, Efrosinibarbayianni, George Kokotos, J. Andrew Mccammon, And Edward A. Dennis˙Development Of Potent And Selective Inhibitors For Group VIA Calcium-Independent Phospholipase A_2 Guided By Molecular Dynamics And Structure Activity Relationships. J Med Chem. 2016 May 12; 59(9): 4403–4414.

[5] S Qiu, F Chen, Y Liu, L Lai. Discovery Of Novel Secretory Phospholipase A2 Inhibitors Using Virtual Screen. Chem.Biol. Drug.Des.2014; 84: 216-222

[6] Fortes-Dias CL, Fonseca BC, Kochva E, Diniz CR. Purification And Properties Of An Antivenom Factor From The Plasma Of The South American Rattlesnake (Crotalusdurissusterrificus). Toxicon. 1991;29(8):997–1008.

[7] K Higashino, Y Yokota, T Ono, S Kamitani, H Arita And Khanasaki. Identification Of A Soluble-Form Phospholipase A_2 Receptor As A Circulating Endogenous Inhibitor For Secretory Phospholipase A_2. J Biol Chem. 2002 Apr 19;277(16):13583-8.

[8] Fortes-Dias CL, Lin Y, Ewell J, Diniz CR, Liu TY. A Phospholipase A2 Inhibitor From The Plasma Of The South American Rattlesnake (Crotalusdurissusterrificus). Protein Structure, Genomic Structure, And Mechanism Of Action. J Biol Chem. 1994;269(22):15646–51.

[9] Manoj G. Tyagi, Sumith K. Mathew.Lipoprotein Associated Phospholipase A2 Enzyme; Possible New Roles And Inhibition For Therapeutic Intervention. Int J Res Med Sci. 2014 Aug;2(3):805-809

[10] Dos Santos RM, Oliveira LC, Estevão-Costa MI, De Lima ME, Santoro MM, Fortes-Dias CL. Inhibition Of Crotoxin Binding To Synaptosomes By A Receptor-Like Protein From Crotalusdurissusterrificus (The South American Rattlesnake). Biochimbiophysacta. 2005;1717(1):27–33

[11] Faure G, Villela C, Perales J, Bon C. Interaction Of The Neurotoxic And Nontoxic Secretory Phospholipases A_2 With The Crotoxin Inhibitor From *Crotalus* Serum. Eur J Biochem. 2000;267(15):4799–808.

[12] Fortes-Dias CL, Diniz CR, Kochva E. Neutralization By Homologous Plasma Of Crotalusdurissusterrificus (South American Rattlesnake) Venom And Crotoxin. Ciênc Cult. 1990;42(7):501–6.

[13] Perales J, Villela C, Domont GB, Choumet V, Saliou B, Moussatché H, Bon C, Faure G.Molecular Structure And Mechanism Of Action Of The Crotoxin Inhibitor From Crotalusdurissusterrificus Serum. Eur J Biochem. 1995;227(1–2):19–26.

[14] Dufton, M.J.; Hider, R.C. Snake Toxin Secondary Structure Predictions. Structure Activity Relationships.*J. Mol. Biol.* 1977, *115*, 177–193.

[15] Habermann E, Breithaupt H. Mini-Review. The Crotoxin Complex-An Example Of Biochemical And Pharmacological Protein Complementation. Toxicon. 1978; 16 (1) : 19-30

[16] Alam, M.I.; Alam, M.A.; Alam, O.; Nargotra, A.; Taneja, S.C.; Koul, S. Molecular Modeling And Snake Venom Phospholipase A_2Inhibition By Phenolic Compounds: Structure-Activity Relationship. *Eur. J. Med. Chem.* 2016, *114*, 209–219.

[17] Roberts A (2014) Coronary Artery Disease: Darapladib Fails To Improve The Stability Of CAD. Nat Rev Cardiol 11(6):310.

[18] Huang F, Subbaiah PV, Holian O, Zhang J, Johnson A, Gertzberg N, Lum H(2005) Lysophosphatidylcholine Increases Endothelial Permeability: Role Of Pkcalpha And Rhoa Cross Talk. Am J Physiol Lung Cell Molphysiol 289(2):L176–L185.

[19] Wilensky RL, Shi Y, Mohler ER 3rd, Hamamdzic D, Burgert ME, Li J, Postle A, Fenning RS, Bollinger JG, Hoffman BE, Pelchovitz DJ, Yang J, Mirabile RC, Webb CL, Zhang L, Zhang P, Gelb MH, Walker MC, Zalewski A, Macphee CH.(2008) Inhibition Of Lipoprotein-Associated Phospholipase A2 Reduces Complex Coronary Atherosclerotic Plaque Development. Nat Med 14(10): 1059–66

[20] Wang WY, Zhang J, Wu WY, Li J, Ma YL, Chen WH, Yan H, Wang K, Xu WW, Shen JH, Wang YP. (2011) Inhibition Of Lipoprotein-Associated Phospholipase A2 Ameliorates Inflammation And Decreases Atherosclerotic Plaque Formation In Apoe-Deficient Mice. Plos One 6(8):E23425.

[21] Acharya NK, Et Al. (2013) Diabetes And Hypercholesterolemia Increase Blood-Brain Barrier Permeability And Brain Amyloid Deposition: Beneficial Effects Of The Lppla2 Inhibitor Darapladib. J Alzheimers Dis 35(1):179–198.

[22] Iwase M, Sonoki K, Sasaki N, Ohdo S, Higuchi S, Hattori H, Iida M.(2008) Lysophosphatidylcholine Contents In Plasma LDL In Patients With Type 2 Diabetes Mellitus: Relation With Lipoprotein-Associated Phospholipase A2 And Effects Of Simvastatin Treatment. Atherosclerosis 196(2):931–936.

[23] Staurenghi G, Ye L, Magee MH, Danis RP, Wurzelmann J, Adamson P, Mclaughlin MM. (2015) Darapladib, A Lipoprotein-Associated Phospholipase A Inhibitor, In Diabetic Macular Edema: A 3-Month Placebo-Controlled Study. Ophthalmology 122(5): 990–996

[24] Madej T, Lanczycki CJ, Zhang D, Thiessen PA, Geer RC, Marchler-Bauer A, Bryant SH.MMDB And VAST+: Tracking Structural Similarities Between Macromolecular Complexes.Nucleic Acids Res. 2014 Jan; 42(Database Issue):D297-303

[25] Nargotra, A.; Sharma, S.; Alam, M.I.; Ahmed, Z.; Bhagat, A.; Taneja, S.C.; Qazi, G.N.; Koul, S. In Silicoidentification Of Viper Phospholipasea2 Inhibitors: Validation By In Vitro, In Vivo Studies. J. Mol. Model. **2011**,17, 3063–3073.

[26] R Parvathy, J Sudhaarya , Ajayalekshmy. Moleculardocking Studies Of Bioactive Compounds From Njavara The Medicinal Rice Of Kerala, As Phospholipase A_2 Inhibitors. Int. J. Res. Ayurveda Pharm. 7(Suppl 3), Jul -Aug 2016, 159

[27] Kokotos G, Hsu YH, Burke J, Baskakis C, Kokotos C, Magrioti V, Dennis E. Potent And Selective Fluoroketone Inhibitors Of Group VIA Calcium-Independent Phospholipase A_2. J Med Chem. 2010;53:3602–3610.

[28] Hsu YH, Bucher D, Cao J, Li S, Yang SW, Kokotos G, Woods V, Mccammon J, Dennis E. Fluoroketone Inhibition Of Ca^{2+}-Independent Phospholipase A_2 Through Pinding Pocket Association Defined By Hydrogen/Deuterium Exchange And Molecular Dynamics. J Am Chem Soc. 2013;135:1330–1337.

[29] Wallace, A.C.; Laskowski, R.A.; Thornton, J.M. LIGPLOT: A Program To Generate Schematic Diagrams Of Protein-Ligand Interactions. Protein Eng. Des. Sel. **1995**, 8, 127–134.

[30] Trott, O.; Olson, A.J. Autodockvina: Improving The Speed And Accuracy Of Docking With A New Scoring Function, Efficient Optimization, And Multithreading. J. Comput. Chem. **2010**, 31, 455–461.

[31] A LDe Araújo,François Radvanyi Determination Of Phospholipase A2 Activity By Colorimetric Assay Using A Ph Indicator.Toxicon 25(11):1181-8,1987

[32] Wei-Chiao Chang, Charmaine Nelson, And Anant B. Parekh.Ca^{2+} Influx Through CRAC Channels Activates Cytosolic Phospholipase A_2, Leukotriene C_4 Secretion, And Expression Of C-Fos Through ERK-Dependent And -Independent Pathways In Mast Cells. The FASEB Journal Vol. 2006, 20, No. 13, Pp. 2381-2383.

[33] Jingjingli,Yingxiong, Shimin Sun, Lehan Yu, And Chunhonghuang·Preparation Of Monoclonal Antibodies Against Gamma-Type Phospholipase A_2 Inhibitors And Immunodetection Of These Proteins In Snake Bloodj Venom Anim.Toxinsincl Trop Dis. 2017; 23: 37.

[34] Degroot AS, Sbai H, Varnavas D. Aubin CS, Mcmurry J, Martin W. Immuno-Informatics: Mining Genomes For Vaccine Components. Immunol Cell Biol. 2002;80:255–269.

[35] Mohanapriya M, Nandhini A R, Praveen Kumar P, Yoganandhini G, Gowri Shankar B A.Anti-Phospholipaseactivity Of Medicinal Plants Against Najanaja Venom Anti-Phospholipase.Int.Res.J.Pharm.2017,8(10)-189-195

[36] Stephens D, Barbayianni E, Constantinou-Kokotou V, Peristeraki A, Six DA, Cooper J, Harkewicz R, Deems RA, Dennis EA, Kokotos G. Differential Inhibition Of Group IVA And Group VIA Phospholipases A_2 By 2-Oxoamides. J Med Chem. 2006;49:2821–2828.

[37] Antonopoulou G, Barbayianni E, Magrioti V, Cotton N, Stephens D, Constantinou-Kokotou V, Dennis EA, Kokotos G. Structure-Activity Relationships Of Natural And Non-Natural Amino Acid-Based Amide And 2-Oxoamide Inhibitors Of Human Phospholipase A_2enzymes. Bioorg Med Chem. 2008;16:10257–10269.

[38] Wan, J.; Gong, X.; Jiang, R.; Zhang, Z.; Zhang, L. Antipyretic And Anti-Inflammatory Effects Of Asiaticoside In Lipopolysaccharide-Treated Rat Through Up-Regulation Of Heme Oxygenase-1. Phyther. Res. **2013**, 27, 1136–1142

[39] Singh, R.K., Singh, N., Jabeen, T., Sharma, S., Dey, S., Singh, T.P. (2005) J.Drug Target. **13**: 367-374

[40] Pavan, R.; Jain, S.; Shraddha; Kumar, A. Properties And Therapeutic Application Of Bromelain: A Review. Biotechnol. Res. Int. **2012**, 2012, 1–6.

[41] Gohil, K.J.; Patel, J.A.; Gajjar, A.K. Pharmacological Review On Centellaasiatica: A Potential Herbal Cure-All. Indian J. Pharm. Sci. **2010**, 72, 546–556.

[42] Tohda, C.; Yang, X.; Matsui, M.; Inada, Y.; Kadomoto, E.; Nakada, S.; Watari, H.; Shibahara, N. Diosgenin-Rich Yam Extract Enhances Cognitive Function: A Placebo-Controlled, Randomized, Double-Blind, Crossover Study Of Healthy Adults. Nutrients **2017**, 9, 1160.

[43] Fatahiya Mohamed Tap ,Fadzilahadibahabd Majid ,Hassan Fahmi Ismail ,Tet Soon Wong ,Kamyarshameli ,Mikio Miyake And Nurulbahiyah Ahmad Khairudin·In Silico And In Vitro Study Of The Bromelain-Phytochemical Complex Inhibition Of Phospholipase A2 (Pla2), *Molecules* **2018**, *23*(1), 73

[44] Jiyoungkim,Ki Won Lee, Hyongjoo Lee. Polyphenols Suppress And Modulate Inflammation: Possible Roles In Health And Disease.Chapter 29 –Polyphenols In Human Health And Disease**, 1,** 2014, Pages 393–408

Manoj G Tyagi"Development of Novel Phospholipasea2 Inhibitors Using Molecular and Computational Techniques" IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.2 (2018): 05-12.