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DOCKING OF ROSMARINIC ACID TO INHIBIT T CELL ACTIVATION AND COX-2 mRNA SYNTHESIS AND TO EVALUATE ITS IMMUNOSUPPRESSIVE AND ANTICANCEROUS ACTIVITY

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ABSTRACT:

Rosmarinic acid (RA) is a natural polyphenolic compound found in Lamiaceae family. It has many medicinal properties such as anti-inflammatory, anti-cancerous, anti-allergic, immunosuppressive effects. Organ transplants evoke a variety of immune responses in the host resulting in Host-Versus-Graft rejection and Graft-Versus-Host Disease. Immune responses in transplantation rejection are primarily dependent on T cell, agents that suppress the functions of T cells are likely to be more efficacious drugs. Lymphocyte Cell Kinase (LCK) is a T cell-specific protein tyrosine kinase, which is a key molecule of T cell receptor (TCR) -mediated signaling and plays an important role in both T cell maturation and activation. LCK contains SH2 domain, SH3 domain, and tyrosine kinase domain. Rosmarinic acid has the potential to inhibit the LCK-SH2 domain by binding to its cognate ligand thereby preventing the immune disorders. Expression of Cyclooxygenase-2 (COX-2) has been reported to be elevated in human colorectal adenocarcinoma and other tumors, including Breast, Cervical, Prostate, and Lung Tumors. Pharmacological inhibition of COX-2 has been shown to protect against experimentally-induced carcinogenesis. Hence Rosmarinic acid is docked with LCK-SH2 and signal transduction proteins involved in COX-2 mRNA synthesis such as NF-κB (Nuclear Factor Kappa B), MAPK (Mitogen Activated Protein Kinase) and CREB (Cyclic AMP Response Element Binding protein) using Argus lab (4.0 version). The results shows that Rosmarinic acid has the potential to inhibit proteins LCK-SH2, NF-κB and MAPK but it failed to inhibit CREB.

Keywords: Rosmarinic acid, LCK-SH2 domain, COX-2, NF-κB, MAPK, CREB.

[I] INTRODUCTION

Organ transplantation is now common for patients with end-stage renal, cardiac, hepatic or pulmonary failure. Currently, allografts of kidney, heart, lung, liver, bone marrow, pancreas (Islet cells), cornea, small intestine and skin are routinely performed. The immune response is primarily triggered by T cells through recognition of alloantigen, and the major targets in transplant rejection are non-self allelic forms of class I and class II Major Histocompatibility Complex (MHC) antigens. LCK (Lymphocyte Cell Kinase), a T cell-specific protein tyrosine Kinase, plays key molecule of TCR (T cell receptor)-mediated signaling and plays an important role in both T cell maturation and activation[14]. The protein

contains SH2 domain, SH3 domain, and tyrosine kinase domain [1].

Inhibition of T cell activation and is effectors functions an attractive target for the development of drugs to treat T cell-mediated immunopathologies, like autoimmune diseases as well as transplant rejection[15].

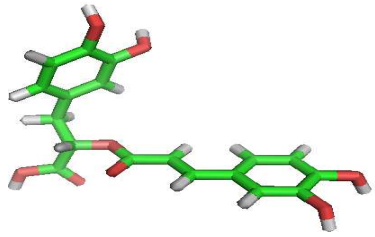
Currently, allograft rejection is controlled using immunosuppressive agents such as cyclosporine A, FK506, azathioprine, corticosteroids including prednisone, Rapamycin and methylprednisolone cyclophosphamide [18].

Although the development of new immunosuppressive drugs has led to substantial improvement in the survival of patients, these drugs are associated with a high incidence of side effects such as nephrotoxicity and/or hepatotoxicity [2]. Thus, there is a need for natural immunosuppressive agents acting specifically on immune cells.

Rosmarinic acid [RA] (MF: $C_{18}H_{16}O_8$) (PUBCHEM ID: CID 5281792) belongs to the group of polyphenols mostly found in Lamiaceae family. (E.g.) *Prunella vulgaris, Perilla frutescens* and *Coleus blumei* [7]. It is disclosed that Rosmarinic acid and derivatives thereof efficiently suppress immune responses induced by the transplantation of animal tissue as well as specifically inhibit the SH2 domain of LCK[3]. Hence it can be used to treat or prevent the diseases or their pathological effects which are mediated by interaction between SH2 domains and their natural ligands, and also used to treat or prevent immune disorder such as organ transplantation rejection, graft-versus-host disease, autoimmune disease and chronic inflammatory diseases. Rosmarinic acid has antioxidant, anti-inflammatory, anti-cancerous, anti-allergic and antimicrobial activities.

Extract of *Perilla frutescens* (PE) or RA appears to be a strong anti-inflammatory and antioxidant agent as it inhibits mast cell release of histamine, inhibits lipoxygenase activity[11]. Reports suggest that the body's response to cancer has many parallels with the inflammatory response. In addition, cyclooxygenase-2 (COX-2), a key enzyme in the inflammatory response, is thought to affect carcinogenesis indirectly via its modulatory effect on the inflammatory response and the immune system.

Fig: 1. 3D Structure of ROSMARINIC ACID:



Rosmarinic acid follows Lipinski's rule of five, (i) Molecular weight: 360.31484 [g/mol], (ii) XLogP3-AA: 2.4, (iii) H-Bond Donor: 5, (iv) H-Bond Acceptor: 8 [13].

Expression of cyclooxygenase-2 (COX-2) has been reported to be elevated in human colorectal adenocarcinoma and other tumors, including those of breast, cervical, prostate, and lung [16]. Genetic knock-out or pharmacological inhibition of COX-2 has been shown to protect against experimentally-induced carcinogenesis [17]. Results from epidemiological and laboratory studies indicate that regular intake of selective COX-2 inhibitors reduces the risk of several forms of human malignancies [20]. Thus, it is conceivable that targeted inhibition of abnormally or improperly elevated COX-2 provides one of the most effective and promising strategies for cancer chemoprevention[19].

[II] MATERIALS AND METHODS

2.1 Materials

2.1.1 Databases and Tools used

The protein databases taken for investigation were **SWISS-PROT** and **NCBI.**

The structural and domain architecture databases used were Protein Data Bank **(PDB)** and Smart Modular Architecture Research Tool **(SMART).**

The structural viewers used were **Swiss-Pdb** viewer and **PyMOL** viewer.

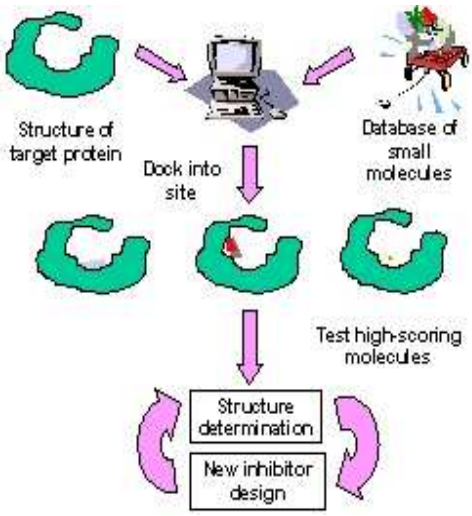
The docking analysis was done using **Argus Lab (4.0 Version).**

2.2 Methodology

1. Retrieval of the 3D structure of proteins LCK-SH2, NF-kB, MAPK, CREB from the PDB (protein data bank) and is saved as PDB file.
2. Conversion of 2D structure of Rosmarinic acid to 3D structure using SMILES format by open Babel.
3. Identification of the active pockets of the receptor from Q-SITE FINDER.
4. Docking of receptor with ligand by Argus lab.
5. Analysis of results.

Fig: 2. Docking Flowchart:

[III]



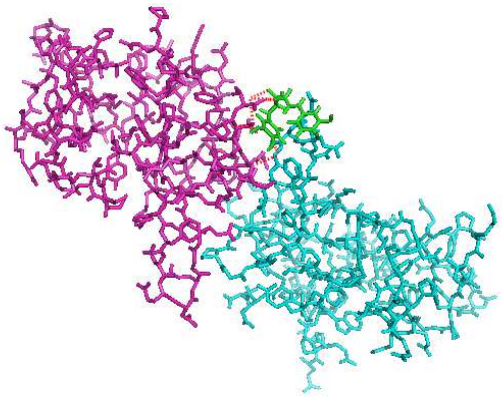
RESULTS

The proteins (LCK-SH2, NF-kB, MAPK, and CREB) are docked with the RA using the protein and ligand docking server ARGUS LAB.

3.1 Argus Lab Result for LCK- SH2

The SH2 domain of LCK protein present in TCR of T cells was docked with the immunosuppressive drug ROSMARINIC ACID **[Figure -3]**. The RA binds with both chain A and chain B of the LCK-SH2 domain. The amino acid ASN (Asparagine) at the 173rd position of the LCK-SH2 was given as the binding site in the Argus lab docking server. The best dock pose energy was -6.53198 kcal/mol.

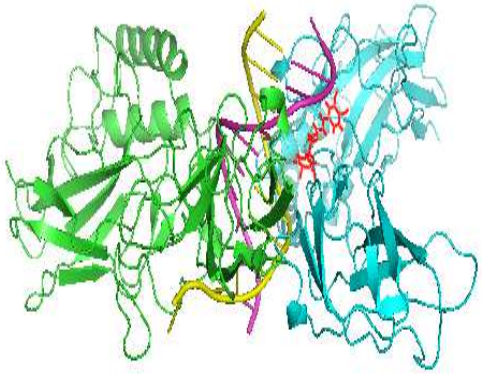
Fig: 3. Argus lab result for the docking of the protein LCK-SH2 with the ligand ROSMARINIC ACID:



3.3 Argus Lab Result For NF-κB

The following picture shows the docking structure of the protein NF-κB (which is transcription factor for the synthesis of COX-2 mRNA) with ROSMARINIC ACID. The amino acid LEU (Leucine) at the 658th position of the protein NF-κB was given as the active site. The best ligand pose energy was found to be -7.96521 kcal/mol.

Fig: 4. Argus lab result for docking between NF-κB and ROSMARINIC ACID:

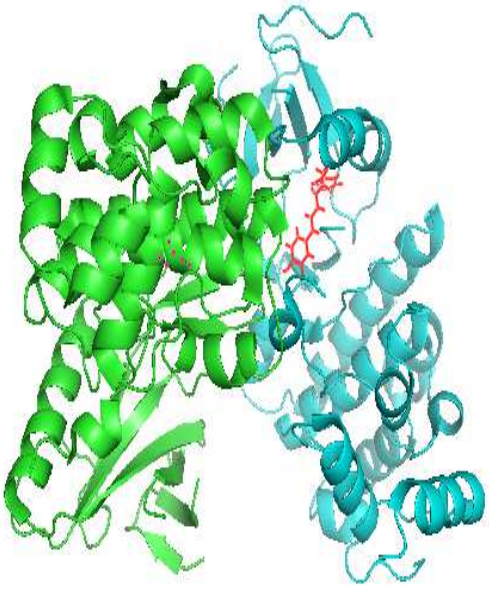


3.4 Argus Lab Result For The Protein MAPK

The following picture shows the docked structure of the protein MAPK with the drug

ROSMARINIC ACID. The amino acid 211 LYS was given as the binding site. The best ligand pose energy was found to be -7.51966 kcal/mol.

Fig: 5. Argus lab result for docking between MAPK and ROSMARINIC ACID:



3.5 Argus Lab Result for the Protein CREB

The result showed that the receptor CREB failed to dock with the ligand Rosmarinic acid. The docking was not completed because of the lack of binding pockets in the receptor (CREB) for the ligand to bind.

[IV] CONCLUSION

Thus the docking studies were carried out to confirm the interaction of the drug Rosmarnic acid with the protein LCK-SH2 and with the signal transduction proteins (NF-κB, MAPK, and CREB) involved in the COX-2 mRNA synthesis. It confirms that, the drug binds with the SH2 domain of LCK which is responsible for T cell activation and inhibits the further phosphorylation of signal transduction proteins in the T cell thereby preventing the graft rejection during organ transplantation and autoimmune diseases. Rosmarinic acid has the potential to inhibit the proteins NF-κB (nuclear factor kappa B), MAPK (mitogen activated protein kinase) but it failed to inhibit CREB (cyclic AMP response element binding protein) which is also involved in the signal transduction pathways of COX-2 mRNA synthesis because it has no affinity towards the receptor. Hence it is concluded that, the Rosmarinic acid is a best natural drug which can be used for the treatment of immune disorders and cancer.

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