In silico interaction of rutin with some immunomodulatory targets: a docking analysis

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The use of plant products as immunomodulator has a long history. Forms eternal era, plant, mineral and animal products are used as drugs for the treatment of various diseases. The present-day synthetic compounds find their leads in natural products. The process of immunomodulation retunes the immune system of an individual by restoring routine functions. Research on immunomodulators from natural sources has been extensively studied for modulation of immune system along with protection and prevention of diseases. Rutin, a flavonoid has been investigated for its potential immunomodulatory effects. Increased particle clearance, decreased inflammation, increased leucocyte and antibody production was observed with administration of rutin in rats. The present study is focused on exploring in silico interaction of rutin with some chemokines and inflammatory targets. In this study, rutin was docked with TNF-α, IL-1β, IL-6, and NOs. Docking studies revealed the excellent interaction of rutin with these targets. The result of present study provides insight for the discovery of novel molecules for immunomodulation and treatment of inflammatory disorders. Findings from the present study show that rutin may interact with several chemokines and inflammatory mediators. Further studies on rutin and associated flavonoids are necessary to develop and establish QSAR and QSPR studies which may serve a stepping stone for the development of novel and safe immunomodulator. Rutin therefore, can be considered for development of an immunomodulatory agent.

Keywords: Docking, Immunomodulatory, Inflammation, Interleukin, Nitric oxide, Prostaglandin

Immune system comprises some immune cells that include lymphocytes, killer cells, macrophages and neutrophils that can prevent body against invading infections. In such event, various interconnected biomolecular pathways lead to inflammation, destruction of microbes and promotion of wound healing process. Innate immunity can be regarded as the first line of defense that comprises of various physical, biochemical and cellular components. Adaptive immunity comprises production of antibodies that protect the body against pathogen invasion (e.g., opsonisation). During inflammation, acute phase aids to fight against the early phase of infection. Chronic inflammation sometimes may lead to progression of autoimmune diseases. In chronic inflammation, over expression of inflammatory cytokines (associated with abnormal gene expression) is observed. Thus, inhibition of these over expressed pro-inflammatory cytokines seems to be beneficial in autoimmune diseases (viz. rheumatoid arthritis). Immuno-active chemicals produced from macrophages play a vital role in inflammation. Macrophages when coming in contact with bacterial endotoxin lipopolysaccharide produce an array of proinflammatory cytokines which chiefly include interleukins (IL-1β, and IL-6), tumor necrosis factor (TNF-α) and other inflammatory mediator viz. prostaglandin E2 (PG-E2) and nitric oxide (NO). In the case of overactive secretion of these mediators, sometimes, cardiovascular diseases, autoimmune diseases, and cancer progression may be observed. Nitric oxide (NO) is a pro-inflammatory mediator that leads to the prognosis of inflammation. Its overproduction is observed in macrophage (stimulated due to lipopolysaccharides) and its metabolites are harmful to cellular integrity.

Pharmaceuticals from natural source have been used by humanity since eternal era to treat various diseases. Chemotherapeutic agents used today have immunosuppressive and cytotoxic effects that prove detrimental to the health of individuals. Thus, it seems to be imperative to search for immunomodulatory agents from natural origin. In addition to immunomodulation, these compounds have antiradical
and antioxidant properties. Flavonoids are polyphenolic compounds found abundantly in plants. They are known to exert analgesic, antidiabetic, antimicrobial and cytotoxic effects. Rutin is ubiquitous flavonoid in plants. The term rutin comes from the plant *Ruta graveolens* L., which is one of the most abundant sources of rutin. Buckwheat, raspberry, blackberry, tomato, fenugreek, tea, and grapes are rich sources of rutin. Rutin is an antioxidant and demonstrated various pharmacological effects on living systems. There are various reports about analgesic and anti-inflammatory effects. Recently, rutin was evaluated for its possible immunomodulatory effects whereby protective effects of rutin on cellular, and humoral effects were seen. Rutin proved itself to be a novel immunomodulator as the treatment with rutin caused a significant increase in humoral antibody titer, increased carbon clearance, and increased leucocyte count. The present study aims to explore underlying mechanism of immunomodulation. In this study, in silico interaction of rutin with various immunomodulatory cytokines was analysed. Along with this, the molecular basis of interaction of rutin with various immunomodulatory targets was determined.

**Materials and Methods**

**Software**

Python 2.7-language was downloaded from www.python.com, Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, Discovery Studio visualizer 4.1 was downloaded from www.accelerys.com.

**Docking**

The three-dimensional crystalline structures of 4 proteins were obtained from Protein Data Bank (http://www.rcsb.org/). These protein were TNF-α (PDB ID: 2AZ5), IL-1β (PDB ID: 2NVH), IL-6 (PDB ID: 1P9M) and NOs (PDB ID: 5U01). The structurally refined protein .pdb files were converted to .pdbqt files using grid module of autodock tools 1.5.6. Charges were assigned to the ions to the proteins manually wherever necessary. The 2D and 3D chemical structures of rutin was retrieved (http://pubchem.ncbi.nlm.nih.gov/). These .sdf and .mol files obtained from PubChem were converted into .pdb files using Marvin Sketch (http://www.chemaxon.com/marvin/sketch/index.jsp). These .pdb files were converted to .pdbqt using ligand preparation module of autodock tools 1.5.6. The docking of rutin was carried out using the Autodock tools (ADT) v1.5.4 and autodock v4.2 programs. Rutin was docked to all the target protein complexes with the molecule considered as a rigid body. The search was carried out with the Lamarckian Genetic Algorithm; populations of 100 individuals with a mutation rate of 0.02 have been evolved for ten generations. The remaining parameters were set as default. The docked structure was then visualised using Discovery Studio 2016 for obtaining the binding interactions.

**Results**

The four crystal structures of proteins were retrieved from protein databank. A docking program was performed to simulate the binding mode to identify the precise binding sites on various immunomodulatory targets. Docking studies were carried out on active sites of five target proteins 2AZ5, 1ITB, 1P9M and 5U01 with rutin. Figure 1-4 indicates the interaction of rutin with the active pocket of immunomodulatory targets which demonstrated minimum binding energy with targets via non-covalent interaction. The docking of rutin with TNF-α showed that it showed hydrogen bonding with the backbone at chain A: Gly-121 while the sugar moiety also showed hydrogen bonding with Chain B: Gly121 and Tyr-121. Beside these major interactions, Flavanol moiety also showed π-amide interaction with Chain B: Gly-121, Gly-122, and π-π interaction with Chain A: Tyr-59. The other minor alky and π-alkyl interactions were observed with ChainB: Leu-57, Leu-94, and Phe-124 respectively (Fig. 1). Its binding affinity was $-5.13$ Kcal/mole.

Rutin also showed interaction with IL-1β. Its polyphenolic flavanol nucleus, as well as sugar units, showed hydrogen bond interaction with Arg-4, Phe-46, Gln-48 and Lys-103. The sugar provides quite strong interaction with Arg-4 with three hydrogen bonds. The Glu-42 also demonstrated π-anion interaction flavanol nucleus (Fig. 2). The presence of such high number of hydrogen bonds makes it potential inhibitors of IL-1β. Further, the docking of rutin with IL-6 showed various H-bond interactions with helix A and D. These helices interact with IL-1α receptors. The Asp-34, Ser-37, Lys-171 and Gln-175 residues have demonstrated multiple H-bond interactions. Apart from these interactions, Leu-33, Arg-30 showed π-σ interactions. Asp-30 showed π-cation and Leu-178 demonstrated π-alkyl interaction (Fig. 3).

Rutin shows various interactions with NOs which include H-bond interaction with Ser-339, Trp-683, Val-685, Asp-601 and Hem-801, π-π stacking with Trp-683, π-σ interaction with Met-341 and π-alkyl interaction with Met-341 (Fig. 4).
Molecular docking is a valid practice which aids to envisage the principal binding modes of the ligand with the protein having known three-dimensional structure. The study focused on binding modes which were necessary for major structural interaction, and provide useful information for designing the inhibitors.

Molecular docking is one of the widely explored techniques which is used to discover potential ligands for known targets. These compounds can be screened based on the estimation of free energy binding. The value of free energy binding signifies the affinity of drug towards the target. Similarly, lowest inhibition constant represents the potential compound\textsuperscript{14}.

**Fig. 1 — Molecular docking studies of rutin against TNF-α [(A) 2D-interactions; & (B) 3D-interactions]**

**Fig. 2 — Molecular docking studies of rutin against IL-1β [(A) 2D-interactions; & (B) 3D-interactions]**

**Discussion**

Molecular docking is a valid practice which aids to envisage the principal binding modes of the ligand with the protein having known three-dimensional structure. The study focused on binding modes which were necessary for major structural interaction, and provide useful information for designing the inhibitors.
In the present study, TNF-α (PDB ID: 2AZ5), IL-1β (PDB ID: 1ITB), IL-6 (PDB ID: 1P9M), and NOs (PDB ID: 1NSI) were analysed for possible interaction with a flavonoid rutin. Cytokines demonstrate a key role in the development of inflammation and tissue destruction leading to progression of inflammatory diseases. Necrosis factors and interleukins are two major classes of cytokines involved in the progression of hyperalgesia. In the present study, TNF-α, IL-1β, and IL-6 were selected for docking analysis. With Rutin, inhibitory constant for TNF-α was 25.62 µM, 61.42 µM with IL-1β, and 12.35 µM. TNF-α is a
key regulator of inflammation. Overactivity of this chemokine is responsible for various inflammatory diseases like rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease. TNFR1 contains a death domain (DD) motif lying towards its C-terminal involved in death signalling while in case of TNFR2 no such death motif is present and works through apoptotic mechanism. TNFR2 have a greater affinity for TNF ligand and is colocalised with TNFR1. It slowly passes ligand to TNFR1 and gets shedded off the membrane while TNFR1 get internalised showing its further action. So, the binding of TNF ligand with TNFR is the critical step. Cunningham et al. showed the complex between the small molecule TNF inhibitor and the intact TNF-α trimer results in a 600-fold accelerated trimer dissociation. The XRD study showed small molecule inhibitor displaces a subunit of the trimer to form an inhibitor bound TNF-α dimer complex. We also docked the rutin on the similar site.

The docking study revealed that rutin mimic similar interaction to that of 6,7-dimethyl-3-[(methyl[2-[methyl[(1-[3-(trifluoromethyl)phenyl]-1h-indol-3-yl]methyl]amino][ethyl]amino)methyl]-4h-chromen-4-one present in actual pdb. The study showed that the rutin at the molecular level might inhibit the trimer assembly of TNF-α which may be its molecular mechanism to prevent inflammation. In a study, rutin pretreatment significantly reduced levels of TNF-α in LPS stimulated animals. Similar protective effects of rutin on glial cells were observed due to rutin pretreatment. Polygala paniculata L. extract (rich in rutin) demonstrated IL-1β inhibition. Similar inhibitory effects were seen in present docking studies.

IL-1β along with IL-6 and TNF-α produces hyperalgesia. During injury, IL-1β promotes expression of Interleukin-6. Drugs against TNF-α and proinflammatory interleukins (IL-1β and Interleukin-6) seems to be useful against psoriasis, Crohn’s disease, rheumatoid arthritis. In a study, rutin demonstrated hepatoprotective effects primarily due to suppression of IL-6 in rat hepatocytes in CCl4 intoxicated animals and high-cholesterol-diet-fed rats. In the present study, docking results revealed affinity of rutin towards IL-6 for the inhibition of later.

Interleukin-1 is a proinflammatory cytokine that induces acute as well as chronic inflammation through expression of the various gene. Interleukin-1 has two isoforms, i.e. IL-1α and IL-1β. It is observed that IL-1β gene spontaneously expressed. So, inhibiting expressed IL-1β may manifest to prevent and cure many inflammatory conditions. IL-1β shows its action by binding through the Interleukin-1 receptor. The Arg-11 and Gln-15 forms contact with domain two while His-30 and Gln-32 form contact with domain 1-2 junction of Interleukin-1 receptor. The seven discontinuous residues, i.e. Arg-4, Leu-6, Phe-46, Ile-56, Lys-93, Lys-103, and Glu-105 together constitute a binding site in IL-1β which bind to Interleukin-1 receptor. Thus, binding of Rutin with some of the crucial residues of this site may describe one of its anti-inflammatory mechanism of action.

IL-6 (IL-6), comprises of four-helix bundle linked by loops with a mini-helix. IL-6 is a secreted from T cells and macrophages which bind to two cell surface receptors, IL-6Rα and gp130. It is involved in several chronic inflammatory diseases. The IL-6 forms association with IL-6α-gp130 leading to the ternary complex which activates the JAK family (tyrosine kinases) and the downstream STAT3 transcription factor. The helices A and D, forms N-terminus to C-terminus, interact with domain-2 and 3 of the IL-6Rα. The interaction of rutin with domain A and D leads to a possible inhibitory effect on its action mediated through, IL-6Rα receptor. Such inhibition over IL-6 is c by previous study.

Nitric oxide synthase is an essential class of enzyme that synthesizes nitric oxide from l-arginine. The Nitric oxide synthase is responsible for NO production from Arginine. Its activity is controlled by Ca2+ concentration through Ca2+-calmodulin complex. The expression of TNF-α, IL-1 follows NOs creation. This enzyme is abundantly found in macrophages and monocytes at the site of infection or inflammatory disease. Thus, due to the biosynthesis of nitric oxide, bacterial growth is prevented, and inflammation is retarded due to suppression of proliferation of T cells. However excessive production of nitric oxide and generation of reactive nitrogen intermediates is involved in the progression of many inflammatory diseases. Thus, inhibition of this enzyme may serve a vital role in the prevention of inflammation. Several natural products have been studied for inhibition of inducible nitric oxide synthase; for instance flavonoids from Tanacetum microphyllum DC, especially Ermanin and 5,3-dihydroxy-4'-methoxy-7-methoxycarbonyl flavonol were most potent inhibitors of this enzyme. In NOs, the arginine urea group forms a bi-dentate interaction with Glu-377 adjacent to the active site. It is a site of larger competitive inhibitors that may inhibit i-NOs. Rutin retained all the primary interaction as...
shown by the co-crystallized NOs inhibitor. This porphyrin ring plays an important role in catalytic enzyme mechanism. In the present study, in silico docking studies revealed inhibition of NOs. Thus the docking interaction of NOs with rutin confirms the previous results whereby rutin exerted protective effects, probably by inhibiting NOs in ischemic reperfusion injury.

Conclusion

In the present study, we carried out docking studies on rutin to various inflammatory and immunomodulatory targets, with the purpose to study and analyze in silico interaction of former on later. The results obtained from docking and study of the interactions of the rutin suggests the ability of rutin to bind to multiple targets involved in inflammation and immunomodulation. Rutin interacted with various chemokines and inflammatory mediators viz. TNF-α, IL-1β, IL-6, and NOs. With each target, rutin demonstrated a noteworthy affinity for binding. Findings from the present study show that rutin may interact with several chemokines and inflammatory mediators. Further studies on rutin and associated flavonoids are necessary to develop and establish QSAR and QSRR studies which may serve a stepping stone for the development of novel and safe immunomodulator.

References


