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Structural modeling and simulation studies of human cyclooxygenase (COX) isozymes with selected terpenes: Implications in drug designing and development

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ABSTRACT

In view of recently implicated role of COX-1 in human health and diseases, including cancer, development of safe and selective drugs, as COX-1 inhibitor is desirable. Human COX-1 and COX-2 isozymes have been modeled using *in silico* tools and relative efficacies of terpenoids as their inhibitors have been investigated by docking. The docking analyses of 10 selected terpenoids along with drugs revealed that all of the terpenoids were more potent inhibitors of COX-1 rather than COX-2 with the oleanolic acid as the most potent inhibitor of COX in general (binding energy [−18.68 Kcal/mol and −18.25 Kcal/mol] and estimated Ki [5.57×10^{-8} μM and 11.4×10^{-8} μM] for COX-1 and COX-2, respectively) and β-carotene as most selective inhibitor of COX-1. Furthermore, ibuprofen and aspirin were found to be preferential inhibitor of COX-1 and COX-2, respectively.

1. Introduction

Cyclooxygenase (COX) catalyzes cyclization and oxygenation of arachidonic acid into prostaglandins (PGs) which are ubiquitous fatty-acid derivatives playing crucial roles in cancer, neuro-inflammation, cardio-protection, pain and fever [1]. In human, mainly two isozymes of COX (COX-1 and COX-2) exist. COX-1 has been reported to be expressed constitutively at low levels in majority of cell and tissue types to high levels in platelets and stomach [2] while, COX-2 has been considered to be primarily an inducible enzyme with many regulatory roles. Thus, COX-2 expressions have been shown to be induced by bacterial endotoxin such as lipopolysaccharide (LPS) and cytokines namely, interleukin, growth factors and tumor necrosis factor-α [2]. Based on these differences, traditionally, it was suggested that the major action of COX-1 is to mediate the gastrointestinal tract protection and modulate platelet function, whereas COX-2 is mainly involved in inflammation and pain. Thus, COX-1 has been suggested to be responsible for the primary prostanoid response to inflammatory stimuli (particularly, in cells and tissues where it is constitutively and predominantly expressed), whereas that of COX-2 has been suggested to be the major contributor to prostanoid synthesis especially in inflammation progress [3–5].

In recent years, a number of reports have appeared which suggest newer roles for COX-1 in human health and diseases including cancer. Involvement of COX-1, in the pathophysiology of diseases, such as pain, fever, analgesia, neuroinflammation and cancer is based on both preclinical and clinical reports proposing COX-1 as a potential therapeutic target [6]. Thus, involvement of COX-1, rather than COX-2, in carcinogenesis has been demonstrated emphatically in ovarian and breast human cancers [7–9]. Moreover, involvement of COX-1 in a number of other type of cancers such as prostate [10], cervical [11], neck and head [12] has also been documented. Furthermore, in a study using selective inhibitor of COX-1 and COX-2 in resident peritoneal macrophages (RPM), it has been demonstrated that secretion of tumor necrosis factor-α (TNF-α) was regulated by PG synthesized primarily by COX-1 [13]. Involvement of COX-1 in neuroinflammation, a key stage in the development of several neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, traumatic brain injury and HIV-associated dementia has been correlated with constitutive level of expression of COX-1 in various areas of brain [14]. Furthermore, involvement of COX-1 in management/treatment of post operative pain processing and sensitization has also been suggested on the basis of COX-1 expression in spinal cord and gracile nucleus [15].

In view of these reports, relevance of COX-1 in comparison to COX-2, in the pathophysiology of these diseases has to be relooked. Many efforts have been made to find out the possible relevance of COX-1 and COX-2, in relation to various pathophysiological conditions, using specific as well as non-specific drugs and inhibitors. Thus, ibuprofen, a commonly used non-streroidal anti-inflammatory drug (NSAID), has recently been shown to inhibit tumor growth in a mouse cancer model by

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preferentially inhibiting COX-1 [16]. On the other hand, aspirin (another NSAID) has been reported to inhibit growth of many types of tumors including breast cancer by preferential inhibition of COX-2 [17]. With regard to mode of action of these two non-steroidal drugs, it is reported that ibuprofen inhibits COX reversibly by competing with arachidonic acid binding at active site while that of aspirin inhibits COX irreversibly by acetylating a serine residue at the active site [18,19]. These NSAIDs suffer from the disadvantages of having side effects. The long-term treatment of these NSAIDs namely aspirin, ibuprofen, piroxicam, mefenaminic acid etc. causes gastrointestinal damage with development of ulcers and bleeding. Furthermore, due to regular use of NSAIDs the production of PGs is reduced that causes the reduction in the rate of glomeruleric filtration leading to fluid retention, hypertension as well as renal failure [20]. Recently, Curhan et al. [21] have also reported that the regular uses of NSAID drug were responsible for hearing loss in the patients. Thus, for therapeutic applications, there is a need for more specific inhibitors of COX coming from natural sources which are relatively safer with little or no side effects. Terpenoids (also referred to as isoprenoids), one of the largest group of secondary metabolites with huge structural diversity, are reported to be present in higher plants, algae, insects, microbes, mosses, liverworts, lichens as well as in marine organisms [22]. Terpenoids constitute minor but ubiquitous components of our diet, and are considered as relatively non-hazardous to humans [23]. They are available as a distinguished resource of pharmacologically important agent with therapeutic applications as anti-inflammatory, anti-bacterial, anti-malarial, anti-cancerous and cardioprotective [22,24]. Thus, oleanolic acid has been reported to possess hepatoprotective, anti-inflammatory and anti-cancerous activities [25]. β-carotene has been shown to possess anti-oxidative, anti-cancerous and cardioprotective activities [26]. α-humulene has been shown to possess anti-inflammatory effect and also inhibits tumor cell growth [27,28]. Curcumin has wide therapeutic actions such as anti-inflammatory, anti-microbial, anti-cancerous, hepatoprotective and neuroprotective [29]. Zinger contains many terpenes and their derivatives such as zingiberene, β-bisabolene and sesquiphellandrene, have been shown to possess chemopreventive property [30]. More specifically, zingiberene has recently been shown to exhibit anti-oxidative, anti-cancerous, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-hyperlipidemic and anti-emetic actions [31]. Black pepper which contains terpenoids like α-pinene and sabinene have been shown to have anti-viral, anti-inflammatory properties [32]. Farnesene has been shown to possess anti-inflammatory effect [33].

In the present paper 10 terpenoids namely, α-humulene, α-pinene, β-bisabolene, β-carotene, curcumin, farnesene, oleanolic acid, sabinene, sesquiphellandrene, and zingiberene have been analyzed through molecular modeling approaches for their therapeutic potentials, with reference to inhibition of COX isozymes, in the pathophysiology of indicated human diseases.

2. Materials and methods

2.1. Modeling of COX-1 and COX-2

To the best of our knowledge no human COX models are available on the PDB, therefore, in the present study both isoforms of human COX have been modeled. Primary sequences of COX-1 and COX-2 were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/, Swiss-Prot ID: P23219.2, P35354, respectively). Program Modeller 9v8 was performed to build the 3D structures of COX-1 and COX-2 according to the homology modeling method using ovine prostaglandin H2 synthase-1(PDB ID: 1q4g) as template. The obtained template and COX-1 and COX-2 sequences were imported into the ClustalW program (www.ebi.ac.uk/Tools/msa/clustalw2/) for the determination of sequence similarity. Energy minimizations of each of five generated models were done by offline tool SPDV [34]. Necessary changes to targets were done using UCSF Chimera [35].

Steriochemical validations of the models were done using PROCHECK tool (http://nihserver.mbi.ucla.edu/SAVES/). These structures were evaluated by ERRAT (http://nihserver.mbi.ucla.edu/ERRATv2/) tool which provided the graphical presentation of overall quality factor of the models. Validated models were submitted to Protein Model Database (PMDB) as homology models (ID No: PM0077520 for COX-1 and PM0078073 for COX-2).

Superimposition of the validated models of COX-1 and COX-2 were done using UCSF Chimera [35].

2.2. Preparation of ligands structure

Based on available literature, terpenoids with therapeutic properties such as anti-inflammatory, anti-bacterial, and anti-cancerous were selected. Accordingly, 10 naturally occurring terpenoids

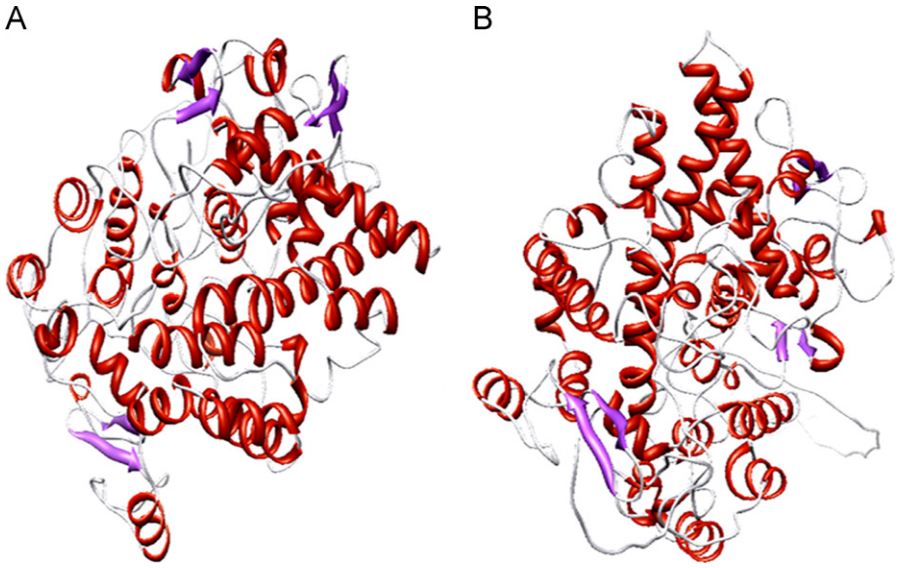

Fig. 1. The final considered model of human cyclooxygenase-1 (A) and cyclooxygenase-2 (B).