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Clinical Implications of Basic Sciences

Molecular Docking helps in understanding the action of Paracetamol (acetaminophen): an approach towards finding a better COX2 inhibitor.

M. Asif Qureshi,[1] Taha Junaid Khan,[2] M. Saquib Qureshi[3]

Dow University of Health Sciences and University of Karachi, Pakistan

ABSTRACT

Objective

To test paracetamol binding to various COX isoforms in order to identify its potential use as a COX2 inhibitor.

Methods

We used Bioinformatics to perform molecular docking of paracetamol with various COX-isoforms.

Results

There was strongest binding of paracetamol with COX2 (E-value = -165.9) as compared to COX1 (E-value = -160.9) and COX3 (E-value = -149).

Conclusion

We propose paracetamol as a potential COX2 inhibitor, a hypothesis that is worth testing to override the side effects caused by using currently available COX2-inhibitors. We, therefore, highlight the necessity of further studies to explore the use of paracetamol as an effective COX2-inhibitor. (Rawal Med J 2011;36:237-240).

Key words

COX2 inhibitor, drug designing, molecular docking, paracetamol.

INTRODUCTION

Paracetamol (acetaminophen or n-acetyl-p-aminophenol) is a very commonly used analgesic and antipyretic. Even after more than a century of its discovery, debate still exists about how paracetamol exerts its therapeutic actions.[1] Although several mechanisms (including serotonergic and NO inhibition) have been proposed but inhibition of cycloxygenases (COXs) is considered to be the main mechanism of paracetamol action.[2-4] Studies have suggested a generalized inhibitory potential of paracetamol for COX activity.[5,6] In humans, various isoforms of COXs have been identified till date including COX1, COX2 and COX3. COX1 is supposed to have homeostatic role.[7] COX2 is an inducible form and its expression is increased in pathological conditions including inflammation, fever and cancer.[8]

COX3, an alternative splice variant of COX1, is thought to be specifically inhibited by paracetamol in central nervous system.[9] Whether or not COX3 acts as a prostaglandin synthetase and contributes towards pathogenesis of pyrexia remains controversial.[9] Involvement in several pathologies brings forward COX2 as a very important therapeutic target. Several COX2 inhibitors have been designed till date including non steroidal anti-inflammatory drugs (NSAIDs) which are broadly classified into selective and non selective

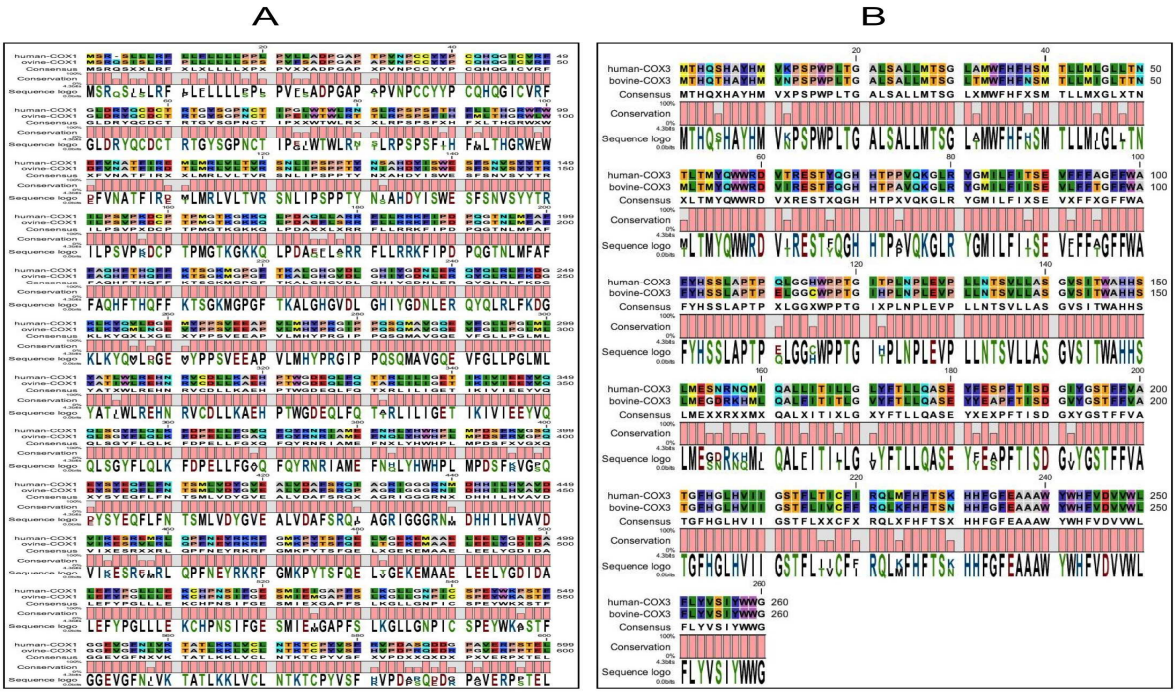
COX inhibitors. Non selective NSAIDs inhibit both COX1 and COX2 and, therefore, interfere with the homeostatic function of COX1 resulting in severe side effects such as gastrointestinal ulcers (GI) ulcers. Selective NSAIDs include COX2 specific inhibitors (the "coxibs"). They spare COX1 inhibition, nevertheless, they are also associated with adverse effects such as thrombosis and myocardial dysfunctions.[10] Various researchers are now turning their interests towards exploring the role of paracetamol as a potential COX2 inhibitor and emphasis is being made upon conducting more studies to elucidate its role in effective COX2 inhibition.[11]

Bioinformatics has recently emerged as an extremely powerful tool which helps in various fields of science and technology. It has revolutionized the basis of drug designing. Molecular docking is one of the available tools in Bioinformatics and it has contributed tremendously in drug designing not only because it saves precious time but also it provides great precision and accuracy and thus contributes tremendously in the various areas of biological sciences.[12,13] The aim of this study was to determine if paracetamol binds to COX2 effectively.

MATERIALS AND METHODS

Amino acid sequence retrieval and alignment: Amino acid sequences (primary structures) of human-COX1 (protein ID: P23219), ovine-COX-1 (protein ID: P05979), human-COX2 (protein ID: P35354), human-COX3 (protein ID: PQ9b2U6) and bovine-COX-3 (protein ID: Q6QTG5) were retrieved from Uniprot Database (www.uniprot.org). Uniprot is a central protein repository which is universally used for accessing protein information.[14] Structural homologues of these proteins were searched using BLAST tool at NCBI database (www.ncbi.nlm.nih.gov). Amino acid sequences were aligned and analyzed using software called CLC genomics workbench 5 and Clustal X.[15]

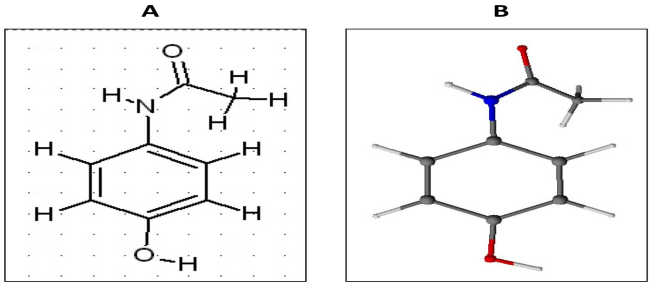
Fig 1. Multiple sequence alignments of (A) human-COX1 and ovine-COX1, (B) human-COX-3 and bovine-COX3. Conservation percentages within the sequences are illustrated as histograms.



Tertiary structures of COX-1, COX-2 and COX-3: Tertiary structures of human-COX1 (COX1) and human-COX3 (COX3) were constructed using SWISS-MODEL online server of Swiss Institute of Bioformatics (http://swissmodel.expasy.org). SWISS-MODEL is a protein homology modelling server and is commonly used for modelling tertiary structures of proteins by using templates of closely related homologues.[16] For human-COX1 homology modelling, crystal structure of ovine COX1 (PDB ID: 1Q4G) and for human-COX3, crystal co-ordinates of bovine heart cytochrome c oxidase (PDB ID: 1V54) were used as templates,

due to highly conserved sequences (Fig 1). Template crystal structures for homology modelling were retrieved from Protein databank (www.pdb.org).

Fig 2. Structure of paracetamol (acetaminophen): (A) chemical structure retrieved from Drugbank,(B) 3D structure constructed using ACDCLABS 12.0 Chemsketch and 3D viewer.



PDB is an international repository which contains experimentally determined structures of proteins, nucleic acids and other biological molecules.[17] All tertiary structures were viewed and analyzed for various parameters (structural and thermodynamic stability) by using DeepView/Swiss-PdbViewer[18] and Accelrys Discovery Studio 2.5.[19] Stability of the constructed tertiary structures were analyzed both structurally and thermodynamically.

Structure of paracetamol: Chemical structure of paracetamol (primary accession no: DB00316) was retrieved from DrugBank (www.drugbank.ca). The chemical structure was

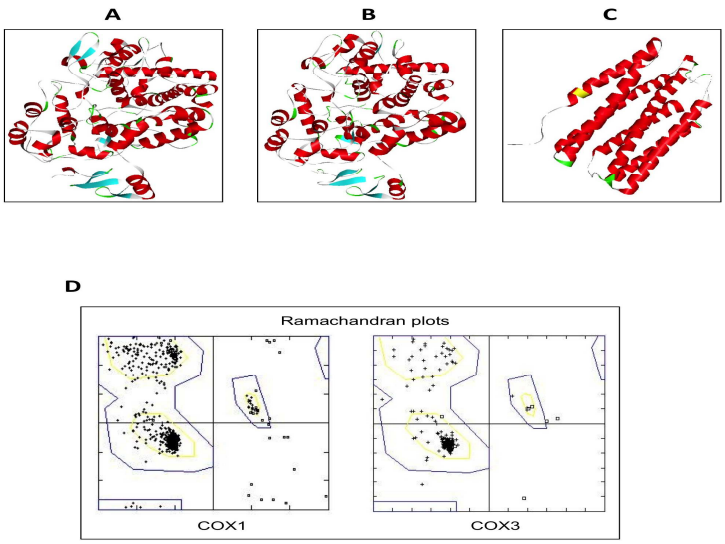
viewed in ChemSketch[20] and its 3D structure was constructed and viewed using 3D-Viewer, both these programmes are parts of a software called ACDLABS version 12.0

Molecular docking of paracetamol with COX1, COX2 and COX3: For docking studies of paracetamol with COX1, COX2 and COX3, docking software Hex version 6b was used. Using this software, we calculated molecular dynamics and thermo stability of docked molecules using free energy simulations. We also calculated and compared energy values (E-values) of the docked molecules to find out the best substrate of paracetamol. There is increasing data in the literature, which supports the use of such techniques for understanding and designing drug molecules.[20]

RESULTS

Forecfiled energy values for both the molecules were in negative (E = -29435.3 KJ/mol for COX1 and E = -11356.82 KJ/mol for COX2), showing that the structures are stable. It was observed that more than 95% of the amino acids were present in the allowed regions for Phi and Psi angles. There were only few amino acids that were present in the disallowed regions but more than 99% of them were glycine residues that because of its neutrality and smallest size, doesn't significantly affect to the structural stability.

Fig 3. Tertiary structures of (A) COX1 (B) COX2, and (C) COX3. These images were created using Accelrys Discovery Studio 2.5.



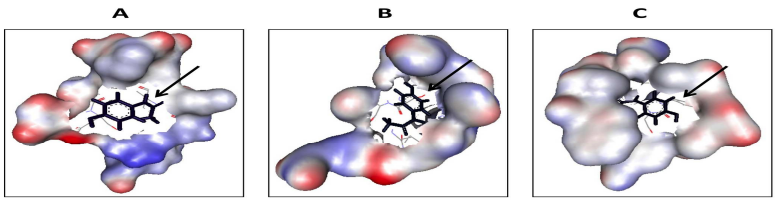
Paracetamol could bind to COX2 more strongly than any other COX isoforms tested (Table 1). The calculated E-values for COX1, COX2 and COX3 were -160.9, -165.9 and -149 respectively.

Table 1. Docking results of COX isoforms with paracetamol.

Enzyme docked with paracetamol	E-value
COX1	-160.9
COX2	-165.9
COX3	-149.0

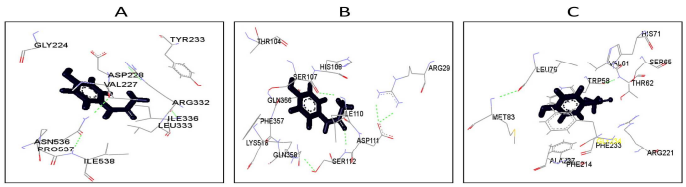
A total of 2000 solutions were analyzed at a grid dimension of 0.6. Out of these, the best poses were selected based on the E- values. These energy values were calculated using Hex version 6b. 3D structure of paracetamol and tertiary structures of COX isoforms used for docking are shown in Fig 2 and Fig 3 respectively.

Fig 4. Possible orientation of paracetamol molecule at binding sites of (A) COX1, (B) COX2 and (C) COX3. Docking was performed using Hex 6b and these images were created using Accelrys Discovery Studio 2.5.



Structural results of molecular docking showing interaction of paracetamol at the binding groove of COX isoforms are shown in Fig 4 and Fig 5.

Fig 5. Possible interacting amino acids of (A) COX1, (B) COX2, and (C) COX3 with paracetamol. These images were created using Accelrys Discovery Studio 2.5.



These images were created using ACDLabs version 12.0 and Accelrys Discovery Studio 2.5.

DISCUSSION

It is clearly suggested by the literature that COX2 is a key player in pathogenesis of several diseases. There is also evidence that almost all of the COX2 inhibitors (both non-selective and selective NSAIDs) are associated with adverse side effects. This brings into account importance of designing a drug which could effectively inhibit COX2 with comparatively less severe side effects. Whether or not paracetamol can serve this job could be a very interesting research area to be explored. Paracetamol is a very commonly used over the counter drug and its interaction with COX2 is quiet vague in the literature.

There are some studies now available in favour of the hypothesis that paracetamol can effectively and selectively inhibit COX2.[11] Using Hex software for molecular docking we showed that COX2 is the most effective receptor for paracetamol binding with lowest E-value of -165.9. Key amino acids of COX2 that were observed interacting with paracetamol

molecule (proposed active site) include Serine 107, glutamine 356, isoleucine 110, phenylalanine 357 and aspartic acid 111 (Fig 5). It appears that these amino acids create a groove where the paracetamol molecule interacts. These finding are very interesting because they makes us think to investigate the use of paracetamol as COX2 inhibitor by performing further experimental studies. As it is well established that currently used COX2 inhibitors (including NSAIDS and others) have adverse side effects, any drug that can potentially replace them with less severe side effects would be a great achievement in pharmaceutics.

CONCLUSIONS

Our molecular docking results showed in this study propose paracetamol as a potential COX2 inhibitor, a proposal that provides an avenue for future research. We, therefore, suggest further in vitro and in vivo studies to test if paracetamol can replace adverse effect causing COX2 inhibitors.

[1.] Research Associate, Dow University of Health Sciences

[2.] Surgical Resident, Dept of Surgery, CHK, Dow University of Health Sciences

[3.] D. Pharmacy 4th year student. Dept of Pharmacy, University of Karachi, Pakistan.

Correspondence: Dr Muhammad Asif Qureshi. Current address: University of Glasgow, Davidson Building, level 4, room 412, Glasgow, G12 8QQ, UK.

Email: m.qureshi.1@research.gla.ac.uk Tel: +44 141 3306229

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