Interaction of acetaminophen and caffeine towards cyclooxygenase-2 (COX-2) in inhibition of prostaglandin (PGH₂) synthesis

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Abstract. Pain is a response of inflammation and can lead to several problems such as morbidity. Inflammation mediated by prostaglandin, which is produced by arachidonic acid and catalyzed by cyclooxygenase. Cyclooxygenase which is responsible for inflammation is COX-2. Acetaminophen can inhibit prostaglandin synthesis through COX-2 and caffeine known as an adjuvant. Analysis of interaction was done by in silico. Ligands, acetaminophen (CID1983) and caffeine (CID2519), was prepared by Discovery Studio v.16 and PyRx then reacted toward COX-2 (1CVU) using PatchDock. Interaction of protein and ligands and energy binding analysed by Discovery Studio v.16 and PatchDock respectively. Energy binding of caffeine towards COX-2 acetaminophen complex is the highest and COX-2 with acetaminophen is the lowest. Interestingly, acetaminophen bind to COX-2 active site, although with the lowest energy and its position is shifted by caffeine when caffeine binds to COX-2 acetaminophen complex. All ligands bind to COX-2 inhibition sites, Ser 530; Tyr 385; Val 523, resulting inhibition of COX-2. Although caffeine can modulate COX-2 inhibition, the combination of caffeine can lead to several gastrointestinal problems. Inhibition of COX-2 can be done by acetaminophen only and it more harmless, besides acetaminophen can bind towards COX-2 active sites with or without caffeine.

1. Introduction

Pain is a response to tissue damage or inflammation [1]. According to the data from the Health Department of Semarang, pain is one of serious issue and the number reaching until 20,294 and it is on 7th in morbidity rank [2]. Inflammation itself is an immune response of infection or tissue damage and affect prostaglandin synthesis. The prostaglandin mediates response of inflammation but also plays a role in homeostasis. The production of prostaglandin is influenced by cyclooxygenase. Cyclooxygenase consists of homodimer COX-1 and COX-2, but COX-2 is responsible in response of inflammation. Cyclooxygenase catalyze conversion of arachidonic acid to prostaglandin, thus inhibition of cyclooxygenase can downregulate synthesis of prostaglandin [3,4,5].

Acetaminophen is an analgesic and anti-inflammatory drug working on the COX pathway by inhibiting COX to produce prostaglandin [6]. Acetaminophen is a weak inhibitor of COX (COX-1/COX-2) but more selective to COX-2. It proves with IC₅₀ value 25.8 ± 1.8 µmol/L of COX-2
whether 113.7 ± 17.2 µmol/L for COX-1. The result is lower for COX-2, thus acetaminophen had proven to be COX-2 selective inhibitor [7]. The analgesic effect of acetaminophen can modulate by caffeine. Caffeine is an adjuvant, so it can increase anti-inflammatory and analgesic effect of acetaminophen itself. [8]. Increasing the effect is caused because caffeine acts as a non-selective adenosine binding with adenosine receptor, but caffeine is a non-selective antagonist of adenosine A_1- and A_2-receptors. Adenosine A_2a receptors induce intracellular signalling events, then upregulating COX-2 gene and releasing prostaglandin E_2 (PGE_2). Thus caffeine can inhibit COX-2 and decrease production of prostaglandin [9]. The binding site occurring the inhibition of COX-2 is still unknown, so through in silico research, we want to prove or encourage the theory. Energy binding and ligands interaction to COX-2 are compared each other and differentiate interaction of COX-2 with acetaminophen alone or combination of acetaminophen and caffeine.

2. Material and Methods

2.1 Ligand Preparation
Acetaminophen (CID1983) and caffeine (CID2519) ligands were downloaded from http://pubchem.ncbi.nlm.nih.gov/ as 3D-SDF format, then energy was minimized and converted to PDB format by Open Babel in PyRx.

2.2 COX Protein Receptor Preparation
COX-2 protein as receptor was downloaded from www.rcsb.org/pdb/ with PDB ID: 1CVU then the protein was prepared by Discovery Studio v. 16 to remove ligands and water molecules.

2.3 Docking of Ligand-Protein and Visualization
Docking were done by PatchDock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) to predict possible interaction and energy binding of COX-2 with acetaminophen and caffeine [10]. Docking was done in order, COX-2 + acetaminophen (CA), COX-2 acetaminophen + caffeine (CAC), and COX-2 caffeine + acetaminophen (CCA). The output of the docking was visualized using Discovery Studio v. 16 to perform the interaction.

3. Result and Discussion
Energy binding of acetaminophen towards COX-2 (CA) is -144.30 kcal/mol and that energy is lower compared to combination of acetaminophen and caffeine. The energy binding of CCA and CAC respectively, are -151.65 kcal/male and 214.31 kcal/Mol (Table 1). From the energy binding result, we can infer that acetaminophen does not bend easily with COX-2 when caffeine initiates the interaction and those ligands are selectively bound to the receptor. Although caffeine binds easily to receptor and improves the energy interaction, acetaminophen can bind with COX-2 with or without caffeine. Besides the energy binding, effectivity and the mechanism of action cannot be concluded only by its energy, thus we need to identify the interaction occurring in the receptor.

Table 1. Energy binding of COX-2 with acetaminophen and caffeine

| Sample                              | Energy(kcal/mol) |
|-------------------------------------|------------------|
| COX-2 + acetaminophen               | -144.30          |
| COX-2 acetaminophen + caffeine      | -214.31          |
| COX-2 caffeine + acetaminophen      | -151.65          |

Interaction of COX-2 with ligands were identified based on their amino acid residues, category of interactions, and type of interactions. Red color in the table is a donor, while the black one is the acceptor. Acetaminophen binds in COX-2 active sites (Table 2), Ser 530; Gly 526; Ala 527; Val 349; Leu 352, and it proves that acetaminophen can inhibit prostaglandin synthesis through COX-2.
Preventing prostaglandin synthesis through COX-2 can be done with complex of acetaminophen and caffeine.

Figure 1. Interaction ligands and receptor. A) Interaction of acetaminophen towards COX-2, B) Interaction of caffeine with COX-2 acetaminophen complex, C) Interaction of acetaminophen with COX-2 caffeine complex
Addition and caffeine cause different interaction too in COX-2 as a receptor (Table 3 and 4), white is the interaction between COX-2 with acetaminophen, while green is the interaction between COX-2 with caffeine. Both interaction has a different amino acid residue. From the result, it can predict if the interaction occurrence also depends on the order of interaction.

### Table 2. Interaction of COX-2 with acetaminophen

| Name                      | Distance   | Category      | Types                      | From Chemistry | To Chemistry |
|---------------------------|------------|---------------|----------------------------|----------------|--------------|
| :LIG1: H – A:SER530:OG    | 2.72612    | Hydrogen Bond | Conventional hydrogen bond | H-Donor        | H-acceptor   |
| A: GLY526: C, O:ALA527: N :: LIG1 | 4.47155 | Hydrophobic   | Amide-Pi Stacked           | Amide          | Pi-Acceptor  |
| :LIG1 – A:VAL349          | 5.16312    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 – A:LEU352          | 5.35157    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |

### Table 3. Interaction of caffeine with COX-2 acetaminophen complex

| Name                      | Distance   | Category      | Types                      | From Chemistry | To Chemistry |
|---------------------------|------------|---------------|----------------------------|----------------|--------------|
| :LIG1: H – A:SER530:OG    | 2.72612    | Hydrogen Bond | Conventional Hydrogen Bond | H-Donor        | H-acceptor   |
| :LIG1: H - B:VAL2349:O    | 2.63263    | Hydrogen Bond | Carbon, Hydrogen Bond      | H-Donor        | H-Acceptor   |
| A: GLY526: C, O:ALA527: N :: LIG1 | 4.47155 | Hydrophobic   | Amide-Pi Stacked           | Amide          | Pi-Orbitals  |
| :LIG1: C - B:VAL2349      | 3.64741    | Hydrophobic   | Alkyl                      | Alkyl          | Alkyl        |
| B:TRP2387 :: LIG1: C      | 4.58875    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 - A:VAL349          | 5.16312    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 – A:LEU352          | 5.35157    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 - B:VAL2349         | 4.64058    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 - B:LEU2352         | 4.9321     | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 - B:VAL2349         | 5.41152    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 - B:LEU2352         | 5.35157    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |
| :LIG1 - B:VAL2523         | 4.58059    | Hydrophobic   | Pi-Alkyl                   | Pi-Orbitals    | Alkyl        |

Caffeine bind to COX-2 active sites which is owned by acetaminophen when acetaminophen is reacted alone to COX-2. In figure 1 acetaminophen is positioned in COX-2 active site, but when caffeine were added (CAC reaction), the position of acetaminophen changed and the acetaminophen binding site in the previous become caffeine binding site (Fig. 2). The binding site of caffeine becomes different too when acetaminophen added later (CCA reaction). Acetaminophen does not shifted caffeine position, but bind beside the caffeine binding site (Figure 3). Interaction of acetaminophen and caffeine occur in Ser 530, Tyr 385, and Val 523, interestingly, it corresponds with the theory stating several COX-2 active sites are Ser 530, Tyr 385, and Val 523 [11, 12, 13, 14,15].

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Catalytic domain mostly constitutes COX-2 active sites and site for substrate binding and NSAIDs action. Entrance of the COX active site occurs at the base of membrane binding domain and leads to hydrophobic channel extending deeply into the interior of the catalytic domain. COX channel can form a constriction (Arg 120, Tyr 355, and Glu 524) and narrowing at the interface between membrane binding domain and catalytic domain. Three amino acid residues act as a separator from the COX-2 lobby with the COX-2 active site. COX-2 active sites located about the constriction and bordered by Val 523 [14, 16].

Interaction of COX-2 with caffeine and acetaminophen occurs in the active sites of COX-2 and inhibition sites of COX-2, Ser 530 and Tyr 385, forming hydrogen bond and hydrophobic bond respectively. The most important amino acids in active sites of COX are Tyr 385 and Ser 530. These amino acids are responsible for inhibition of COX. Hydrogen bond forming in COX Ser 530 and NSAIDs interaction will lead to irreversible inhibition, while the hydrogen bond in COX Tyr 385 and NSAIDs interaction will inhibit the activity of COX [12].

Energy binding and ligands interaction can help to determine the inhibition occur in COX-2. Reaction of caffeine bind to COX-2 acetaminophen complex have high energy binding compare to others, but in this case caffeine may substitute acetaminophen role as a COX-2 inhibitor. Acetaminophen does not bind to its active sites, but caffeine does. Then, the COX-2 inhibition effect might caused by caffeine. We can infer that caffeine has higher selectivity to COX-2, compared to acetaminophen.

Caffeine plays a role as an acetaminophen adjuvant modulating the binding energy and interaction. A combination of two drugs may have side effects, but in this case, a side effects combination of acetaminophen and caffeine in some doses may lead to several gastrointestinal problems [8, 17]. Thus, acetaminophen is better consumed alone without caffeine because it can bind with or without caffeine and interact with the COX-2 active site. Even this research proves a lot of acetaminophen and caffeine interactions towards COX-2 and the inhibition sites, this research need further research with another method.

Table 4. Interaction of acetaminophen with COX-2 and caffeine complex

| :LIG1: H - | Distance | Category | Types | From Chemistry | To Chemistry |
| A:MET522:O | 2.15041 | Hydrogen Bond | Carbon, Hydrogen Bond | H-Donor | H-Acceptor |
| :LIG1: H - | 2.74605 | Hydrogen Bond | Carbon, Hydrogen Bond | H-Donor | H-Acceptor |
| A:VAL349:O | 5.31274 | Hydrophobic | Alkyl | Alkyl | Alkyl |
| :LIG1: C - A:LEU384 | 4.51019 | Hydrophobic | Alkyl | Alkyl | Alkyl |
| :LIG1: C - A:MET522 | 5.4469 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
| A:PHE381 : :LIG1: C | 5.20355 | Hydrophobic | Alkyl | Pi-Orbitals | Alkyl |
| A:TYR385 : :LIG1: C | 5.46566 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
| :LIG1 - A:VAL349 | 5.34958 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
| :LIG1 - A:VAL349 | 5.32177 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
| :LIG1 - A:LEU352 | 4.88283 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
| :LIG1 - A:VAL523 | 4.65172 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
| :LIG1 - A:ILE345 | 5.48249 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
| :LIG1 - A:VAL349 | 4.60697 | Hydrophobic | Pi-Alkyl | Pi-Orbitals | Alkyl |
4. Conclusion
Acetaminophen and caffeine can bind with a COX-2 active site with different energy needed. Caffeine can modulate and improve interaction of acetaminophen towards COX-2 but from the interaction determination, it predicts if acetaminophen is better to consume alone.

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References
[1] Demir Y 2012 Non-Pharmacological Therapies in Pain Management Gabor Racz (Ed) Pain Management- Current Issues and Opinions (Rijeka: InTech)
[2] Dinas Kesehatan Semarang 2016 Profil Kesehatan Kota Semarang 2016 (Semarang: Dinas Kesehatan Kota Semarang)
[3] Streicher J M and Wang Y 2008 Cardiovascular & Hematological Agents in Medicinal Chemistry 6 69-79
[4] Sellers R S, Radi A and Khan N K 2010 Veterinary Pathology 47 601-613
[5] Ricciotti E and FitzGerald G A 2011 Arterioscler Thromb Vasc Biol 31 986-1000
[6] Turtle E J, Dear J W & Webb D J 2013 Br J Clin Pharmacol 75 1396-1405.
[7] Hinz B, Cheremina O and Brune K 2008 FASEB J 22 383-390
[8] Derry C J, Derry S and Moore R A 2012 Cochrane Database of Systematic Reviews DOI: 10.1002/14651858.CD009281.pub2.
[9] Fiebich B L, Lieb K, Hull M, Aicher B, van Ryn J, Pairet M and Engelhardt G 2000 Neuropharmacology 39 2205-2213
[10] Schneidman-Duhovny D, Inbar Y, Nussinov R and Wolfson H J 2005 Nucl. Acids. Res. 33 363-367
[11] Zarghi A and Arfaei S 2011 Iranian Journal of Pharmaceutical Research 10 655-683
[12] Ibrahim M M, Elsaman T and Al-Nour M Y 2018 International Journal of Medicinal Chemistry 2013 1-11
[13] Kiefer J R, Pawlitz J L, Moreland K T, Stegeman R A, Hood W F, Gierse J K, Stevens A M, Goodwin D C, Rowlinson S W, Marnett L J, Stallings W C and Kurumbail R G 2000 Nature 405 97-101
[14] Blobaum A L and L J Marnett 2007 J Med Chem 50 1425-1441
[15] Raharjo S J, Mahdi C, Nurdiana N, Kikuchi T and Fatchiyah F 2014 Advance in Bionformatics 2014 850628
[16] Rouzer C A and Marnett L J 2009 Journal of Lipid Research DOI 10.1194/jlr.R800042-JLR200
[17] Diener H C, Pfaffenrath V, Pageler L, Peil H and Aicher B 2005 Cephalalgia 25 776-787