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A computational prospect to aspirin side effects: Aspirin and COX-1 interaction analysis based on non-synonymous SNPs

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ABSTRACT

Aspirin (ASA) is a commonly used nonsteroidal anti-inflammatory drug (NSAID), which exerts its therapeutic effects through inhibition of cyclooxygenase (COX) isoform 2 (COX-2), while the inhibition of COX-1 by ASA leads to apparent side effects. In the present study, the relationship between COX-1 non-synonymous single nucleotide polymorphisms (nsSNPs) and aspirin related side effects was investigated. The functional impacts of 37 nsSNPs on aspirin inhibition potency of COX-1 with COX-1/aspirin molecular docking were computationally analyzed, and each SNP was scored based on DOCK Amber score. The data predicted that 22 nsSNPs could reduce COX-1 inhibition, while 15 nsSNPs showed increasing inhibition level in comparison to the regular COX-1 protein. In order to perform a comparing state, the Amber scores for two Arg119 mutants (R119A and R119Q) were also calculated. Moreover, among nsSNP variants, rs117122585 represented the closest Amber score to R119A mutant. A separate docking computation validated the score and represented a new binding position for ASA that acetyl group was located within the distance of 3.86 Å from Ser529 OH group. This could predict an associated loss of activity of ASA through this nsSNP variant. Our data represent a computational sub-population pattern for aspirin COX-1 related side effects, and provide basis for further research on COX-1/ASA interaction.

1. Introduction

Aspirin (acetylsalicylic acid, ASA), the most widely used nonsteroidal anti-inflammatory drug (NSAID) has been prescribed for over 100 years, because of its analgesic, antipyretic and anti-inflammatory properties (Singh and Triadafilopoulos, 1999). Taking a daily dose of ASA would reduce the risk of heart attack and stroke as well as many age associated diseases (Antithrombotic Trialists' Collaboration, 2002). ASA irreversibly inhibits cyclooxygenase enzyme (COX) or prostaglandin endoperoxide synthase (PGHS) by acetylating a serine residue at position 529 (human COX residue numbering through this paper), and places a bulky substituent on serine oxygen, which inhibits binding of arachidonic acid (Scheme 1) (Roth and Majerus, 1975; Dewitt et al., 1990). COX enzyme with a molecular weight of 72 kDa is a homodimeric, membrane-bound, hemoprotein and glycoprotein that is present in large amounts in the endoplasmic reticulum of prostanoid-forming cells (Smith, 1986). COX catalyses two separate cyclooxygenase (converting arachidonate to PGG2) and peroxidase (PGG2 go through a two-electron reduction to PGH2) reactions (Gierse et al., 1995), which only the cycloocygenase activity of the enzyme inhibited by ASA.

There are two isozymes of COX that catalyze the first committed step in prostaglandin synthesis: COX-1 is constitutively expressed in most tissues and blood platelets (Funk et al., 1991; Simmons et al., 1991). It produces cellular housekeeping functions of prostaglandins (Smith, 1989). On the other hand, COX-2 (which shares 62% amino acids identity with COX-1) is an inducible form, which is expressed during inflammation (Xie et al., 1991; Fletcher et al., 1992). ASA inhibits both isozymes of COX, but with a greater potency for COX-1 (Blobaum and Marnett, 2007).

Besides its therapeutic effects, nearly 25% of individuals NSAIDs show some type of side effects, such as gastrointestinal dysfunctions (Bloom, 1988). In a study in United Kingdom, researchers indicated that, the responsible drug for over 60% of the deaths caused by adverse drug reactions is ASA (Pirmohamed et al., 2004). Variable drug response and side effects between individuals is attributed to genetic variations in genes that code for drug-metabolizing enzymes, drug transporters, or drug targets. The ASA therapeutic effects are achieved by COX-2 inhibition, nevertheless COX-1 inhibition leads to side effects (Crofford, 1997).

Through personalized medicine strategies, researchers conducted series of experimental studies to classify ASA targets DNA polymorphism based on their effects on drug response or

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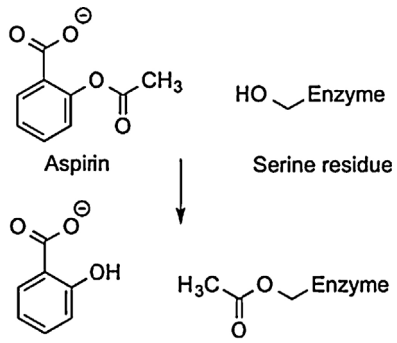

Scheme 1. COX-1 enzyme acetylation by aspirin.

reactions. For instance, Halushka et al. reported that A-842G/C50T (P17L) haplotype was in association with increased potency of ASA as compared with the common allele based on linkage analysis (Halushka et al., 2003). To date, there is no complete information regarding the importance of the side effects of ASA and individual's genotype variability. This study was undertaken to clarify the impact of COX-1 gene SNPs which could alter the amino acids on ASA/COX-1 interaction. The data could indicate that among the nsSNPs examined, individuals with a particular nsSNP can experience therapeutic effects of ASA with a minimal side effects. However, for determining the exact genetic background of the individuals in response to ASA all the examined nsSNPs should be genotyped.

2. Materials and methods

The humanized crystal structure of COX-1 was performed by mutagenizing the residues differing between human and sheep, and then was regularized with BuildModel command of FoldX version 3b5.1 (Guerois et al., 2002; Schymkowitz et al., 2005). Applied amino acid changes are summarized in Table S1 (see Supplementary material).

In order to simulate the favorable position and orientation of intact ASA for acetylating Ser529, flexible docking of ASA in a monomer of COX-1 was accomplished with UCSF DOCK program version 6.4 (Allen et al., 1979; Kuntz, 1992). The basic requirement for docking in DOCK6.4 is a complex crystal structure of ASA/COX-1. Therefore, the co-crystallized ligand of 1Q4G (alpha-methyl-4-biphenylacetic acid) was replaced with ASA using guided ligand replacement (GLR) tool of PHENIX program version 1.7.2-869 (Adams et al., 2010).

All SNPs of COX-1 gene were obtained from dbSNP version 2013.8.16 (http://www.ncbi.nlm.nih.gov/snp/) and only nsSNPs were chosen for further analysis. To configure the PDB files of protein variants containing specific nsSNP, the BuildModel command of FoldX was executed to mutagenize the amino acid of an nsSNP site. Finally, the predicted binding affinity between ASA and each protein variant with DOCK AMBER score was described using AMBERDOCK tool of DOCK6.4 (Graves et al., 2008), which calculates the binding free energy of protein variants with the given ASA position-orientation docking output.

3. Result and discussion

ASA is a widespread used medicine with many beneficial effects that made tremendous excitement for investigators to eliminate ASA side effects, and develop selective COX-2 inhibitors. The therapeutic effects of ASA are carried out by COX-2 inhibition. However, COX-1 is also the primary target of ASA, due to its structural similarities to COX-2, and therefore, experiencing side effects by the individuals who take ASA. Our aim of this investigation was to bring prescribing of ASA into a personalized medicine perspective, and to prepare a subpopulation pattern based on nsSNP polymorphisms data of COX-1. This could elucidate that which individual (genotype) would take advantage of using aspirin in association with showing no or minimal side effects. Because of limited information in this area, a detailed computational study was performed to elucidate COX-1 structural modeling.

3.1. COX-1 structural modelling

The proper human COX-1 3D coordinate for our computational analysis is not available in Protein Data Bank (PDB; http://www.rcsb.org/pdb/). As an alternative way, we obtained the human COX-1 (Swiss Prot accession no. P23219) amino acids sequence from SWISS-PROT protein sequence data bank (http://www.expasy.org/sprot/) and aligned using NCBI's protein-BLAST program to find the homologous sequences (http://www.ncbi.nlm.nih.gov/BLAST/). The most similar sequence structure to human COX-1 is *Ovis aries* (sheep), which shares 96% sequence identity among all available PDBs. For this sequence the best resolved structure was 1Q4G at 2 Å

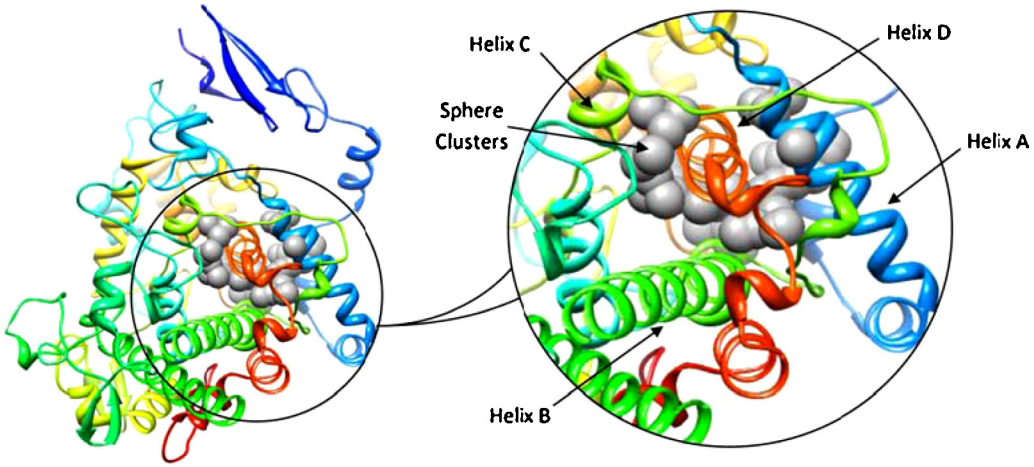

Fig. 1. Representation of 54 of 80 sphere clusters within the cyclooxygenase active site of COX-1. Cyclooxygenase active site lies at the end of a 25 Å hydrophobic channel, which surrounded by four alpha helices named A–D in this figure. These four alpha helices are formed by residues 105–122 (helix A), 324–352 (helix B), 378–383 (helix C) and 519–534 (helix D). The sphere clusters were selected based on PHENIX simulated ASA position.