Celecoxib: A Promising Clinical Dilemma is Associated with Single Nucleotide Polymorphisms

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Abstract—*People are prescribed drugs all the time and different individuals responses to a particular drug differently. The main cause of these different responses is due to single nucleotide polymorphisms. Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic properties. It is approved for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute pain. We have made an attempt to study celecoxib and its interaction with sixty seven genes involved in the prostaglandin (PG) synthesis, apoptosis and angiogenesis metastasis to hit upon the gene mutations which gave rise to different responses to the same drug. Eight of these exon mutations changed the originally coded amino acid with a new one (PTGS1, PTGS2, PTGDR, PLA2G4A, CYP2C9, TBXAS1, TBXA2R and VEGFA). These polymorphisms lie in the extracellular, cytoplasmic, signal sequence and trans-membrane domains of the protein, α-helical regions and β-pleated sheets. These are significant pertaining to the transformation in polarity and charge of the amino acid side chains that in turn modify their interactions with other amino acid residues and aqueous surroundings. This alters the normal functioning of the genes and finally leads to differential responses to the drug celecoxib.*

1. INTRODUCTION

The growth and function of an individual is in great fraction controlled by genes. Mutations can lead to modifications in the structure of an encoded protein or to the reduced or complete loss in its expression. Since a change in the DNA sequence have an effect on all copies of the encoded protein, mutations can be particularly harmful to a cell or organism. A mutation in one DNA nucleotide (SNP, single-nucleotide polymorphism) can prevent a gene from turning into a protein. These seemingly small differences can have very massive consequences, such as how a person responds to a prescription drug. Prescription drugs interact with signaling pathways in our bodies in very specific manner. If the protein that the drug interacts with has been mutated in such a way that the drug cannot interact with it or if the protein is not being made at all, the drug may not function, as it should. Celecoxib is a NSAID that is used to treat osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, painful menstruation, and acute pain. It is also used to reduce the number of colon and rectum polyps in patients with familial adenomatous polyposis. Worldwide, it is estimated that more than 30 million people receive NSAIDs daily. They are one of the most commonly used classes of medicine. Several NSAIDs (aspirin, ibuprofen, and naproxen) are available over-the-counter, but stronger doses and other types of NSAIDs, such as celecoxib, are only available via prescription. It is thought that approximately 25% of the population has experienced NSAID-related side effects that require medical care. Most NSAIDs inhibit both types of cyclooxygenase, PTGS1 (COX-1) and PTGS2 (COX-2) [1]. These enzymes catalyze pathways that play a key role in the generation of the inflammatory response; however, celecoxib selectively inhibits PTGS2.

There are 67 Genes involved in the Pathway

AKR1C3	AKT1	ATP2A1	ATP2A2	ATP2A3	BCAR1	CA12	CA9
CACNA1A	CACNA1B	CACNA1C	CACNA1D	CACNA1E	CACNA1F	CACNA1G	CACNA1H
CACNA1I	CACNA1S	CACNA2D1	CACNA2D2	CACNA2D3	CACNA2D4	CACNB1	CACNB2
CACNB3	CACNB4	CASP3	CASP9	CDKN1A	CDKN1B	CTNNB1	DDIT3
HPGDS	IGFBP3	KCNQ1	KCNQ2	KCNQ3	KCNQ4	KCNQ5	MMP9
PDK1	PLA2G2A	PLA2G4A	PPARG	PTGDR	PTGER1	PTGER2	PTGER3
PTGER4	PTGES	PTGFR	PTGIR	PTGIS	PTGS1	PTGS2	TBXA2R
TBXAS1	VEGFA	ADH1A	ADH1B	CYP2C9	CYP2D6	CYP3A4	IL23R
ALOX12	CTNNB1	SERCA					

The CYP2C9 gene encodes an enzyme involved in the metabolism of many drugs, and is one of the main enzymes that metabolizes and inactivates celecoxib.

2. METHODOLOGY

The Pharmacogenics Knowledgebase, a pharmacogenics resource was used for noting information about the drug celecoxib including its generic or trade (brand) names, its chemical structure, the conditions in which it is used and its general effect on the human body [2, 3]. Pharmacogenics of the drug was understood, which explains how the alleles (versions) of a gene (or genes) that a person has inherited changes its personal biology in a way that makes a drug more

or less effective. The gene alleles which are involved in the pathway were noted and information about their variable response to the drug was gathered and recorded in the form of a table. SNP database of the NCBI website was used for this purpose [4]. rsID of the alleles of interest was searched for, that gave information about the allele of interest. It was further noted whether the allele is an intron or an exon. If the mutation was in an exon, then, recorded the corresponding 'allele change' for each gene. Alleles may also arise from mutations in parts of the gene other than exons i.e. 'NearGene' mutations were also noted. For each allele with a mutation in an exon, the "Amino Acid Sequence Change" was noted.

3. RESULTS AND DISCUSSION

On the basis of prior knowledge of the biological pathways of celecoxib activity, we studied genes involved in: prostaglandin synthesis: PTGS1 (COX1), PTGS2 (COX2), and prostaglandin E2 synthase (PGES) genes, as well as those encoding the epidermal growth factor receptor (EGFR) and thromboxane synthase (TBXAS), genes encoding enzymes of the arachidonate and leukotriene synthesis pathways (PLA2A, ALOX12), interleukin23 (IL23R), and C-reactive protein (CRP), as well as genes involved in EGFR signaling (SRC, EP2, EP4), the prostaglandin dehydrogenase gene (PGDH) and the ornithine decarboxylase gene (ODC1). Clinical manifestations associated with some of these genes are:

PTGS1: Prostaglandin-endoperoxide synthase (fatty acid cyclooxygenase; PGH synthase) is the key enzyme in prostaglandin biosynthesis. The cyclooxygenase activity of the enzyme is inhibited by non-steroidal anti-inflammatory drugs (NSAID). PTGS1 catalyzes the conversion of arachidonic acid to PGH_2, the immediate substrate for a number of cell specific prostaglandin and thromboxane synthases. This occurs via a two-step process, in which the first step introduces two molecules of oxygen to arachidonate, forming the bicyclic peroxide intermediate, prostaglandin G_2 (PGG_2). The second step occurs in a distinct reactive site located on the other side of the molecule, and requires the diffusion of PGG_2 to this site. Here, peroxidation results in the reduction of PGG_2 to the freely diffusible PGH_2. Though these enzymes are membrane bound, they do not contain transmembrane domains; rather, they possess four amphipathic helices juxtaposed such that they form a localized region of hydrophobicity. The hydrophobic region serves to anchor the lower portion of the enzyme in the membrane. The cyclooxygenase active site is located in an area of hydrophobicity near the amphipathic helices. Access to this site occurs via a channel buried in the lipid bilayer. Both substrate and inhibitors use this channel to reach the active site. Polymorphism at position 17 changes Proline to Leucine, this region forms a part of the signal sequence of the protein. Leucine likes to be buried in hydrophobic cores and likes to be placed in alpha helices, whereas, Proline introduces a kink in alpha helices as already discussed. Single nucleotide polymorphisms (SNPs) give rise

to non-conservative amino acid changes in the signal peptide Proline to 17 Leucine of cyclooxygenase-1[5].

PTGS2: PTGS2 (COX-2) is an inducible isoform of COX enzymes that converts arachidonic acid to prostaglandins, which are potent mediators of inflammation. PTGS2 is related to several biological processes, including carcinogenesis, cell proliferation, angiogenesis, and mediating immune suppression. A growing body of evidence has shown that increased expression of PTGS2 is closely related to malignant progression. Moreover, it is reported that selective PTGS2 inhibitors could prevent carcinogenesis. Polymorphism at position 511 changes Valine to 511 Alanine alter the cyclooxygenase active site and creates an even larger hole, which may allow the binding of unusual polyunsaturated fatty acids, other than arachidonic acid [6].

PTGDR: Receptor for prostaglandin D2 (PGD2). Coupled to the G(i)-protein. Amino acid polymorphism at position 190 from Glutamic acid to Lysine lies in the extracellular domain of the protein. Glutamate prefers sharply turning regions on the surface of the protein and is negatively charged whereas lysine is positively charged [7].

PLA2G4A: Cytosolic phospholipase A2 selectively hydrolyzes arachidonyl phospholipids releasing arachidonic acid. It hydrolyzes arachidonic acid from cellular membrane phospholipids, thereby providing enzymatic substrates for the synthesis of eicosanoids, such as prostaglandins and leukotrienes. It is implicated in the initiation of the inflammatory response also. Serine to Proline polymorphism is situated at position 111 in an alpha helix, where Proline might introduce a kink in the helix, thus, destabilizing the structure. However, Proline is often mimicked by Serine due to its smaller structure; it is often found within tight turns [8].
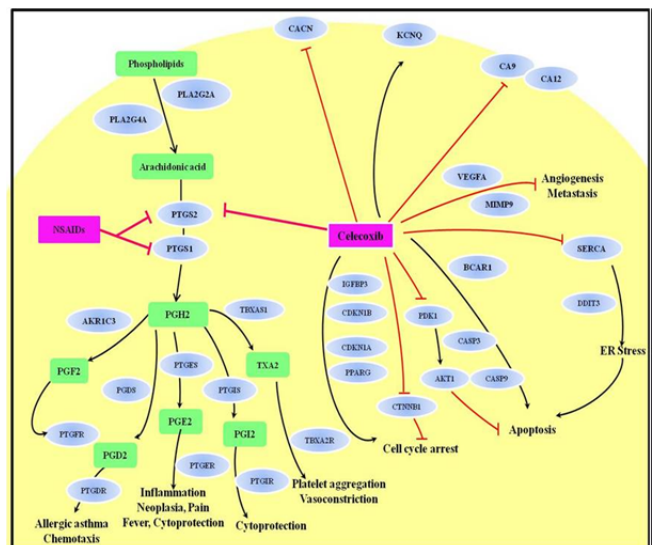

Fig. 1: Stylized cell depicting the mechanism of action of celecoxib and candidate genes interacting with celecoxib and involved in the proposed anticancer mechanisms of celecoxib, including induction of apoptosis, cell cycle arrest, regulation of angiogenesis, and induction of endoplasmic

reticulum (ER) stress. CACN: L-type calcium channels; KCNQ: voltage-gated potassium channels; MMP9, metalloproteinase; NSAIDs, nonsteroidal anti-inflammatory drugs; PGH2, prostaglandin H2; PGE2, prostaglandin E2; PGI2, prostacyclin; PGD2, prostaglandin D2; PGF2, prostaglandin F2; PTGER, prostaglandin E receptors; SERCA, sarcoplasmic/ER calcium ATPases; TXA2, thromboxane A2; VEGFA, vascular endothelial cell growth factor.

CYP450: The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity. CYP2C9 metabolizes approximately 15% of clinically used drugs, and atypical metabolic activity caused by genetic variants in the CYP2C9 gene can play a major role in adverse drug reactions. At least 16 different NSAIDS are metabolized, in part, by CYP2C9. Celecoxib is extensively metabolized by CYP2C9, with minor contributions from CYP3A4, CYP2C8 and CYP2C19. Polymorphism at position 144 and 359 changes Arginine to Cysteine and Isoleucine to Leucine are associated with significantly reduced enzyme activity. Arginine is positively charged polar amino acid, whereas, Glutamine is neutral. Arginines are also frequently involved in salt-bridges where they pair with a negatively charged Aspartate or Glutamate to create stabilizing hydrogen bonds that can be important for protein stability. Arginines are reasonably common in protein active or binding sites. The positive charge means that they can interact with negatively-charged non-protein atoms. However, a neutral amino acid in its place can void off interactions that were otherwise important. Patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin) should be administered celecoxib with caution [9, 10].

TBXA2R: Receptor for thromboxane A2 (TXA2), a potent stimulator of platelet aggregation. The activity of this receptor is mediated by a G-protein that activates a phosphatidylinositol-calcium second messenger system and activates phospholipase C. Thromboxane A2 is an arachidonate metabolite that is a potent stimulator of platelet aggregation and a constrictor of vascular and respiratory smooth muscles. TXA2 has been implicated as a mediator in diseases such as myocardial infarction, stroke, and bronchial asthma [11].

Table 1: The gene alleles involved in platelet aggregation pathway and the SNP's that affect their DNA and RNA sequence, thereby altering the final product formed and the interaction with Celecoxib. Only exon mutations with reported sequence changes and/or reported effects are presented.

GENE	rsID	EXON/ INTRON	CODON SEQUENCE CHANGE (DNA)	CODON SEQUENCE CHANGE (mRNA)	AMINO ACID CHANGE	EFFECT ON AMINO ACID	LOCATION
PTGS2	rs5273	EXON	GTT=GCT	GUU=GCU	Val=Ala	Changes from one nonpolar amino acid to another nonpolar amino acid.	Chromosome 1 q25.2-q25.2
	rs5273	EXON	GTT=GGT	GUU=GGU	Val=Gly	Changes from one nonpolar amino acid to another nonpolar amino acid.	Chromosome 1 q25.2-q25.2
PTGS1	rs3842787	EXON	CCG=CTG	CCG=CUG	Pro17Leu	Polar, uncharged amino acid changes to nonpolar amino acid.	Chromosome 9 q32-q32.3
PTGDR	rs139745811	EXON	GAC - AAG	GAC – AAG	Glu190lys	Negatively to positively charged	Chromosome14 q22.1
PLA2G4A	rs 121434634	EXON	TCT - CCT	UCU – CCU	Ser111Pro	Polar to Nonpolar	Chromosome 1 q25
CYP2C9	rs1799853	EXON	CGT=TGT	CGU=UGU	Arg 144Cys	Positively charged amino acid changes to polar,uncharged amino acid.	Chromosome 10 q23.33-q23.33

	rs1057910	EXON	ATT=CTT	AUU=CUU	Ile 359Leu	Changes from one nonpolar amino acid to another nonpolar amino acid.	Chromosome 10 q23.33-q23.33
TBXAS1	rs13306050	EXON	CTT - CCT	CUU – CCU	Leu82,487Pro	Nonpolar to Polar Uncharged amino acid.	Chromosome 7 7q34-q35
TBXA2R	rs 34377097	EXON	CGC - CTC	CGC - CUC	Arg60Leu	Positively Charged to Nonpolar	Chromosome 19 p13.3
VEGFA	rs2010963	5'UTR	GGT-TGT	GGU-UGU	Gly634Cys	Changes from one nonpolar amino acid to Polar, uncharged	Chromosome 6 6p21.1

A mutation in this gene results in a bleeding disorder. Arginine to Leucine polymorphism at position 60 is brought about a single change in the codon from G to T, this region lies in the cytoplasmic domain of the protein. The Leucine side chain is very non-reactive and is thus rarely directly involved in protein functions like catalysis, whereas, Arginine frequently pairs with a negatively charged Aspartate or Glutamate to create stabilizing hydrogen bonds that can be important for protein stability. This conversion thus may lead to destabilizing the protein structure, ultimately altering its function. TXA2 binding is not affected in this SNP but leads to defective interaction with G proteins and impairs phospholipase C and adenylyl cyclase activation.

TBXAS1: Thromboxane A synthase 1 is a endoplasmic reticulum membrane protein catalyzes the conversion of prostaglandin H2 to thromboxane A2, a potent vasoconstrictor and inducer of platelet aggregation. The enzyme plays a role in several pathophysiological processes including hemostasis, cardiovascular disease, and stroke. Alternatively, spliced transcript variants encoding different isoforms have been found for this gene. Genetic variation in TBXAS1 by mutation or polymorphism is associated with reduced platelet ASA responsiveness. The polymorphism is from Leucine to Proline at position 82 and 487 that lies in the transmembrane helical domain and cytoplasmic domains respectively [12].

VEGFA: VEGFA gene is a member of the PDGF/VEGF growth factor family. It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis. VEGFA is a gene comprised of eight coding exons and several alternative spliced forms that maps to chromosome region 6p1.2. Genetic polymorphisms have been identified outside of the coding region in the 5′ and 3′ flanking regions, and these polymorphisms seem to have an influence on gene expression. Polymorphism at position 634 changes Glycine to Cysteine in the 5'-UTR of VEGF affects the protein translation efficiency. Dysregulated VEGF expression has been implicated in the pathogenesis of a number of diseases [13, 14].

4. CONCLUSION

The work focuses on the single nucleotide polymorphisms that cause different people to respond differently to the drug, celecoxib. Sixty seven genes involved in the prostaglandin (PG) synthesis, apoptosis, angiogenesis metastasis were studied. Out of these, eight genes show changes in amino acid sequences, which are PTGS1, PTGS2, PTGDR, PLA2G4A, CYP2C9, TBXAS1, TBXA2R and VEGFA. The mutations in the non-coding region of DNA may not directly affect the functioning of gene per se, but it may be in the middle of instructions for how the gene should be properly turned into mRNA. In PTGS1, single nucleotide polymorphisms (SNPs) from Proline to Leucine at position 17 gives rise to non-conservative amino acid changes in the signal peptide of cyclooxygenase-1. In PTGS2, polymorphism at position 511 changes Valine to Alanine alter the cyclooxygenase active site which allows the binding of unusual polyunsaturated fatty acids, other than substrate: arachidonic acid. In CYP2C9, polymorphism at position 144 changes Arginine to Cysteine and since the positive charge means that they can interact with negatively-charged non-protein atoms. However, a neutral amino acid in its place can void off interactions that were otherwise important and consequently is associated with significantly reduced enzyme activity. In VEGF, polymorphism at position 634 changes Glycine to Cysteine in the 5'-UTR of VEGF affects the gene expression and consequently, the protein translation efficiency. In conclusion, three types of mutations were observed: exon mutations that resulted in amino acid change; exon mutations that change the codon sequence of DNA but did not result in amino acid change; intron mutations and near gene mutations. Out of these, exon mutations that changed the amino acid sequence were found to be of more importance than others as these change the polarity and charge of the amino acid that causes change in its interaction with other amino acids and its aqueous environment, altering the normal functioning of the genes which may be the cause for different responses to the drug celecoxib by different individuals.

5. ACKNOWLEDGEMENTS

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