

Determining the Molecular Interactions of Multiethnic P-glycoprotein Drug Transporter Variants with Cancer Drugs Involved in Chemotherapy-Induced Peripheral Neurotoxicity (CIPN)

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Abstract

Chemotherapy-induced peripheral neurotoxicity (CIPN) is the most common adverse side effect of cancer treatment that can lead to permanent pain and loss of sensations in both hands and feet. There is currently little insight into the prevalence of CIPN. However, cumulative exposure and mixtures of CIPN-causing chemotherapeutics have been indicated to increase the prevalence of CIPN. In the central nervous system (CNS) and peripheral nervous system (PNS) of the brain, the multidrug-resistance transporter P-glycoprotein (P-gp) is the major determinant of drug and xenobiotic uptake. Based on genetic differences from either individual or ethnic origin, P-gp variants in the form of single nucleotide polymorphisms (SNPs) can alter P-gp's protective efflux function and thus affect chemotherapy's effectiveness in cancer patients.

The objective of this study is to determine the molecular interactions of human P-gp with six common CIPN-causing chemotherapeutics (vincristine, vinblastine, cisplatin, oxaliplatin, etoposide, and paclitaxel) and examine the effects of two SNPs (Serine893Alanine and Serine893Threonine) associated with altering P-gp's efflux function. We conducted literature search and created sequence alignments from all published human brain P-gp plus P-gp from other human tissue. With the gene sequence alignments formed, we plan to design primers to be utilized in polymerase chain reaction (PCR) for amplification. Next, we intend to clone, express, and purify three P-gp variants, including S893A, S893T, and wildtype protein. Using an ATPase activity assay, we aim to identify P-gp inhibitors and substrates among the six selected CIPN-causing chemotherapeutics.

The potential implications of this study will help pinpoint therapeutic drug-drug interactions that facilitate accumulation of chemotherapeutics in the brain to enhance CIPN. In addition, it will inform medical co-administration protocols that can decrease the prevalence of CIPN across ethnic groups. The future direction of this study aims to determine if there is a correlation between P-gp SNP isoform expression levels and CIPN across ethnic groups.

Key words: chemotherapy-induced peripheral neurotoxicity (CIPN), P-glycoprotein (P-gp), single nucleotide polymorphism (SNP), inhibitor, substrate, ethnic

Introduction

The brain is highly susceptible to neurotoxic activity depending on the type of substance or agent that is acting on the nerves (Cavaletti & Marmiroli, 2004). Chemotherapeutics administered in chemotherapy are a form of hazardous substances that directly or indirectly produce neurotoxic activity. Although chemotherapy is the predominant treatment for cancer, new evidence suggests that utilizing this treatment can result in an increased risk of chemotherapy-induced peripheral neurotoxicity (CIPN) (Han & Smith, 2013). CIPN specifically targets the hands and feet of cancer patients, which is commonly known as the “stocking and glove” distribution, causing unexplainable pain and a reduction in the quality of their lives (Harichand-Herd, 2014). The extent of how severe the neurotoxic pain is does not only depend on the chemotherapeutic(s) administered, but various other factors, such as the dosage and duration of treatment (Wozniak et al., 2016). Based on their mechanism of action, CIPN-causing chemotherapeutics are divided into several classes, including platinum-based compounds, taxanes, and vinca alkaloids (Corrie, 2011; Ferrier et al., 2013; Han and Smith, 2013; Harichand-Herd, 2014)

Researchers have focused their attention on the physiological role of multidrug-resistant transporters, specifically P-glycoprotein (P-gp). P-gp, also known as ABCB1 or MDR1, is a member of the ATP binding cassette (ABC) family (Wolking et al., 2015). P-gp is responsible for protecting and effluxing chemotherapeutics out of cancer cells and the brain (Giacomini et al., 2010). P-gp expression accounts for drug sensitivity and resistance to exposed anticancer drugs (Robinson & Tiriveedhi, 2020). Research has shown that chemotherapeutic agents have been identified as substrates and/or inhibitors of P-gp. These chemotherapeutics interact directly at the site of action where P-gp transports exogenous and endogenous compounds out of cells and either get effluxed (substrate) or accumulate (inhibitor) at that site (Shukla et al., 2011).

Genetic variability in individuals accounts for differences observed in response to drug exposure and thus results in differences of neurotoxic outcome. Single nucleotide polymorphisms (SNPs) have been identified as a form of gene variation that influences the activity of many transporters (Shukla et al., 2011). Previous studies have identified common SNPs of P-gp showing conflicting results regarding the effects of these SNPs on transporter function. Some SNPs have the ability to alter the function of a protein and some do not (Marzolini et al., 2004).

This article analyzes two common SNPs of P-gp, which are Serine893Alanine (S893A) and Serine893Threonine (S893T) with nucleotide changes of T > G,A. The goal of this study is to characterize six common CIPN-causing chemotherapeutics (vincristine, vinblastine, cisplatin, oxaliplatin, etoposide, and paclitaxel) as substrates and/or inhibitors of P-gp and analyze how S893A & S893T affect P-gp's function when exposed to these chemotherapeutics. We aim to gain a better understanding of how SNPs affect P-gp drug efflux function and determine the inter-ethnic variability.

Methods

Study Features

Three P-gp variants (Wild Type, S893A, and S893T) are incorporated in this study. We will clone, express, and purify the P-gp variants to determine the interactions with six selected CIPN-causing chemotherapeutics (vincristine, vinblastine, cisplatin, oxaliplatin, etoposide, and paclitaxel). For optimization purposes, an ATPase activity assay will be the method used to determine the molecular interaction of the chemotherapeutics with the P-gp variants. We infer that there will be a different effect in the P-gp variants that contain S893A and S893T. These expected effects of S893A and S893T are decreased P-gp expression and increased susceptibility to CIPN.

We intend to test the hypothesis that single compounds and/or binary combinations of these chemotherapeutics can inhibit P-gp, resulting in an accumulation of these compounds in the PNS to stimulate CIPN. Table 1 shows the known co-administered drugs of our six selected CIPN-causing chemotherapeutics that have been utilized in previous studies (Augustine et al., 2018; Canellos et al., 1995; Cavaletti & Marmiroli, 2004; Giannakakou et al., 1998; Grisold et al., 2012; Hu et al., 2019; Nam et al., 1995). The boxes that are marked with an “X” indicate that the cancer drug in the associated row and column have been shown, in previous clinical research, to be combined in chemotherapy treatment.

Table 1: Known CIPN-causing chemotherapy drug co-administrations tested in this study (Augustine et al., 2018; Canellos et al., 1995; Cavaletti & Marmiroli, 2004; Giannakakou et al., 1998; Grisold et al., 2012; Hu et al., 2019; Nam et al., 1995)

	Vincristine	Vinblastine	Cisplatin	Oxaliplatin	Etoposide	Paclitaxel
Vincristine		X	X		X	
Vinblastine	X				X	X
Cisplatin	X			X	X	X
Oxaliplatin			X			X
Etoposide	X	X	X			
Paclitaxel		X	X	X		

Process

Using the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>) for access to published human brain P-gp or P-gp in other tissue, we were able to get a consensus of which gene sequences can be utilized in our study design. In order to obtain the full registry of published human gene sequences of P-gp, the ‘Nucleotide’ and ‘RefSeq’ databases were consulted. The form ‘*Homo sapiens*’ (human) + ‘ABCB1’ or ‘P-gp’ or ‘MDR1’ was entered into the search bar of each database and from there, the published human P-gp sequences were collated with corresponding accession numbers. Along with NCBI, we used the KEGG Pathway database (https://www.genome.jp/kegg-bin/show_pathway?hsa02010), which provides detailed pathway information on ABC transporters for humans (*homo sapiens*). Since ABCB1 is a part of the ABCB subfamily, the KEGG Pathway database provided a single reference gene sequence that was used for our results. Once we found published gene sequences of human P-gp from NCBI and the single reference sequence from the KEGG Pathway database, we were able to import those gene sequences into CLC Sequence Viewer (http://resources.qiagenbioinformatics.com/manuals/clcsequenceviewer/current/index.php?manual=Introduction_CLC_Sequence_Viewer.html). CLC Sequence Viewer is a bioinformatic resource, which was used to create and compile alignments in this study. Our results will show the outcome of the alignments that were formed through this process.

We intend to use the alignments for polymerase chain reaction (PCR) primer design with the 3 P-gp variants (Wild Type, S893A, and S893T). Once primer design has been completed, we

can then clone P-gp from human brain tissue provided by UC Davis' Alzheimer's Disease Center. Using *E. coli* or yeast expression strains, we aim to express the cloned P-gp variants and purify the protein with fast protein liquid chromatography (FPLC) for analysis. Once the 3 P-gp variants have been cloned, expressed, and purified, we plan on identifying which single and binary combinations of the six known CIPN-causing chemotherapeutics are inhibitors and/or substrates of human brain P-gp.

Results

Literature review has shown that vincristine, etoposide, and paclitaxel are substrates of human P-gp (Crivori et al., 2006; Hodges et al., 2011; Waghray & Zhang, 2018). Vinblastine has been identified as a substrate and an inhibitor of human P-gp (Hodges et al., 2011; Raghava and Lakshmi, 2012). However, it remains ambiguous whether the platinum derivatives, cisplatin and oxaliplatin, are substrates and/or inhibitors of P-gp.

Table 2: Classification of known CIPN-causing chemotherapeutics as substrates and/or inhibitors (Crivori et al., 2006; Hodges et al., 2011; Marzolini et al., 2004; Raghava & Lakshmi, 2012; Waghray & Zhang, 2018)

Chemotherapeutic	P-glycoprotein Classification
Vincristine	Substrate
Vinblastine	Inhibitor/Substrate
Etoposide	Substrate
Cisplatin	<i>Unknown*</i>
Oxaliplatin	<i>Unknown*</i>
Paclitaxel	Substrate

*Unknown** indicates no clear evidence to classify the chemotherapeutic as a P-glycoprotein substrate and/or inhibitor.

Table 3: Summary of all human P-gp gene variants found in the NCBI database. A total of 185 partial and full-length gene sequences have been identified. Gene sequences are listed according to the known source organ, tissue, or cell type.

Located in Brain Tissue	Located in Tissue Other Than Brain	Cancer Cell Line	Unknown Type
44	50	46	45

From both the 'Nucleotide' and 'RefSeq' database of NCBI, a total of 185 P-gp gene variants were found. These gene variants varied in regard to their location in the human body and the type (tissue, cancer cell line, or synthetic construct) shown in Table 3. For our study, we focused on gene variants that had a source location relative to the brain, which resulted in 44 P-gp gene variants from brain tissue. The 44 gene variants were then separated into 2 categories whether they were published as partial or complete gene sequences. As a result, only 3 gene variants were identified as complete and were used in comparison to one another in the alignment design using CLC Sequence Viewer. From the KEGG Pathway database, the reference sequence associated with ABCB1 was added to the alignment design. It is important to note that the reference sequence obtained from the KEGG Pathway database does not provide information on where the gene sequence is located in the human body or the type of sequence it is. Figure 1 shows an alignment created with the reference sequence from the KEGG Pathway database and the 3 complete P-gp gene sequences found in human brain tissue from NCBI. The first sequence is from the KEGG Pathway database and the second to fourth sequences are from NCBI. CLC Sequence Viewer allows you to rename the sequence according to personal preference and in this case, I inputted the accession number followed by the source location (associated with the brain) that was provided from information on that specific gene variant.

When aligned with one another, the gene sequences allow for the discovery and identification of any SNPs as well as any conservation between the sequences that benefit primer design. We discovered a total of 4 SNPs (2 synonymous and 2 non-synonymous) in the alignment we created. Figure 1 shows the 2 non-synonymous SNPs found in our alignment. The single nucleotide changes (shown in red) are located at the base pair (BP) positions of 1,199 and 2,677 in humans. Codons, which are three nucleotide base combinations, code for a specific amino acid. At BP position of 1,199, in the first, second, and fourth sequences (NM_001348946, AK290159, and AB208970), a guanine (G) nucleotide base is observed to code for the amino acid serine. In the third sequence of the alignment (BC130424), an adenine (A) nucleotide base codes for the amino acid asparagine. These single nucleotide changes are indicated as single nucleotide polymorphisms (SNPs). The nucleotide changes from G to A at BP position of 1,199 is deemed a SNP known as Serine400Asparagine. At BP position of 2,677, in the first two sequences of the alignment (NM_001348946 and AK290159), a thymine (T) nucleotide base is used as the codon for the amino acid serine. In the last two sequences of the alignment (BC130424 and AB208970), a guanine (G) nucleotide base is observed to code for the amino acid alanine. At BP position of 2,677, the nucleotide change from T to G is a reflection of the SNP named Serine893Alanine.

The 2 synonymous SNPs (not shown in Figure 1) found in our alignment were Glycine412Glycine and Isoleucine1145Isoleucine. In our alignment, Glycine412Glycine resulted as a nucleotide change from T to C at BP position of 1,236. Isoleucine1145Isoleucine was observed at BP position of 3,435 with a nucleotide change from T to C.

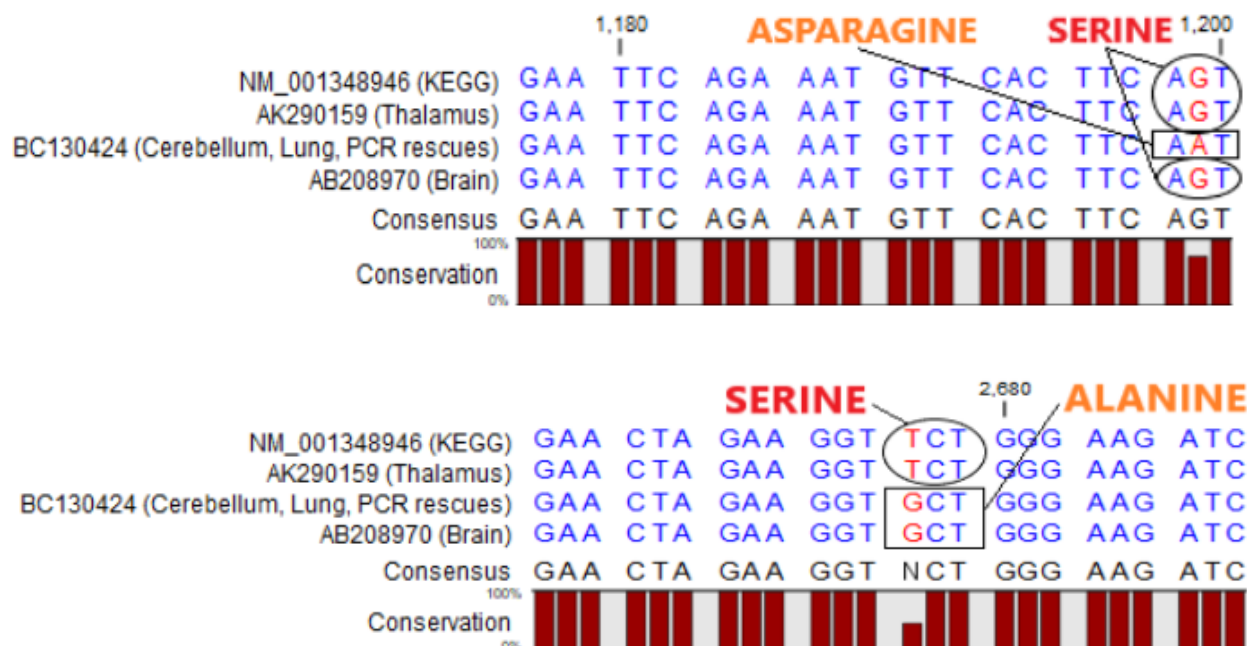


Figure 1: Alignment created from complete P-glycoprotein (P-gp) gene sequences in different brain tissues of the human brain.

Discussion

In clinical settings around the globe, chemotherapy continues to be the leading treatment for cancer. As a result of chemotherapy treatment, many side effects cause cancer patients to endure a poor quality of life. One of the major side effects of chemotherapy is chemotherapy-induced peripheral neurotoxicity (CIPN), which produces painful neurotoxicity amongst cancer patients (Cavalletti & Marmiroli, 2004). Although CIPN is a common side effect of chemotherapy treatment, little is known about how cancer drugs accumulate in the peripheral nervous system to produce this outcome. In our study, we selected six chemotherapeutics known to cause CIPN (vincristine, vinblastine, cisplatin, oxaliplatin, etoposide, and paclitaxel). These chemotherapeutics differ in their mechanism of action but have been shown to interact with P-gp drug transporter function in the brain (Shukla et al., 2011). P-glycoprotein (P-gp) [ABCB1 or MDR1] is an active transporter in the blood-nerve-barrier (BNB) of the peripheral nervous system (PNS) (Saito et al., 2001). P-gp is responsible for effluxing numerous chemotherapeutics out of cancer cells (Leu et al., 1993). Clinical studies have provided evidence that there seems to be multiple factors that influence how chemotherapeutics are able to accumulate. Some of these factors include inhibition of P-gp at the blood-nerve-barrier, decreased expression of P-gp, and the possibility that an unknown mechanism that hasn't been fully studied is responsible (Crivori et al., 2006; Waghray & Zhang, 2018).

In addition, genetic variation accounts for differences observed in individuals, which can alter the function of P-gp in a certain individual. Single nucleotide polymorphisms (SNPs) are the most common class of gene sequence variation, which account for interindividual variability in resistance to cancer drugs. Two common SNPs of P-gp are S893A and S893T (Wolking et al., 2015). These two SNPs have been researched in previous clinical studies and provide evidence of altering drug disposition and drug sensitivity (Marzolini et al., 2004; Wolking et al., 2015).

Previous literature has shown inconsistencies on how P-gp's function alters as a result of SNPs, which is why additional research needs to be completed to bridge these inconsistencies within the literature. A new twist in the literature has shown that these two P-gp polymorphisms are different across ethnic groups (Wolking et al., 2015). The results from our study provide evidence of S893A occurrence in brain P-gp from the comparison of 3 complete P-gp gene variants in the brain obtained from NCBI and 1 reference sequence (unknown location in the human body) from the KEGG Pathway database. Since the KEGG Pathway database and NCBI do not provide information on what ethnic group the gene sequences are from, more research on S893A and S893T needs to be conducted from different ethnic populations to analyze how these P-gp polymorphisms affect P-gp's function when exposed to chemotherapeutics.

Currently, there are no known methods to measure the neurotoxicity that stems from CIPN, but many researchers have formed strategies in hopes to prevent CIPN (Harichand-Herdt, 2014). Unfortunately, there are current disagreements about potential preventative strategies for CIPN. It is essential to understand the extent to which SNPs affect drug uptake and transport activity of P-gp in the brain to help support the creation of new potential preventative strategies. By solidifying which chemotherapeutics in this study design are substrates and/or inhibitors of human P-gp and understanding the impact of S893A/S893T, informed decisions about personalized cancer drug co-administration can be made by medical professionals.

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