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Toluwase Hezekiah Fatoki
Federal University Oye-Ekiti

Omodele Ibraheem
Federal University Oye-Ekiti

Oladoja A. Awofisayo
University of Uyo

Abiodun Samuel Oyedele
Tennessee State University

Olapade Samuel Akinlolu
Federal University Oye-Ekiti

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Toluwase Hezekiah Fatoki[1], Omodele Ibraheem[1], Oladoja A Awofisayo[2], Abiodun Samuel Oyedele[3] and Olapade Samuel Akinlolu[4]

[1]Translational Bioinformatics Unit, Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria. [2]Department of Pharmaceutical and Medicinal Chemistry, University of Uyo, Uyo, Nigeria. [3]Department of Chemistry, Tennessee State University, Nashville, TN, USA. [4]Department of Chemistry, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria.

ABSTRACT: In this study, the interaction of selected pharmaceutical excipients on the function of P-glycoprotein (P-gp) and activity of 6 cytochrome P450 (CYP) isoforms were computationally investigated. At binding free energy cut-off value of −5.0 kcal/mol, the result showed possible modulatory or inhibitory effect by cethyl alcohol on CPY3A4 and P-gp; cetyltrimethyl-ammonium bromide (CTAB) on CYP1A2 and P-gp; dibutyl sebacate on CYP2C9, CYP2E1, and P-gp; sodium caprylate on CYP1A2 and CYP3A4; while most of the tested excipients have good interaction with the cytochromes and P-gp. The predicted pharmacokinetics provided possible inhibitors of the CYPs and P-gp and suggested that aspartame and acetyl tributyl citrate may not permeate blood–brain barrier and not act as P-gp substrates. Target prediction for CTAB showed 100% and 35% probability of target to dynamin-1 (UniProt ID: Q05193) and histamine H3 receptor (UniProt ID: Q9Y5N1), respectively, whereas tricaprylin showed 40% probability of target to 5 Protein kinase C (UniProt IDs: P17252, Q02156, Q04759, P24723, and P05129). This study shows that synergistic effect of some excipients present in a drug formulation and multiple drugs administration is possible through modulation of CYPs activities and P-gp function, and this is crucial for consideration to mitigate toxicity in pediatric and adult populations.

KEYWORDS: Excipients, cytochrome P450, P-glycoprotein, molecular docking, pharmacokinetics

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CORRESPONDING AUTHOR: Toluwase Hezekiah Fatoki, Translational Bioinformatics Unit, Department of Biochemistry, Federal University Oye-Ekiti, PMB 373 Oye-Ekiti, Ekiti State, Nigeria. Email: hezekiahfatoki@gmail.com; toluwase.fatoki@fuoye.edu.ng

Introduction

Pharmaceutical excipients play important role in the overall kinetics of active pharmaceutical ingredients (APIs) by influencing the activity of hepatic and intestinal metabolic enzymes and transporters. The systemic bioavailability of API administered through oral route is often decreased by first-pass biotransformation through both hepatic and intestinal enzymes[1] and may be increased through formulation with excipients that can positively alter the enzymes and transporters responsible for first-pass metabolism. The International Pharmaceutical Excipients Council (IPEC) defines excipients as substances other than the API which have been appropriately evaluated for safety and are intentionally included in a drug delivery system. Pharmaceutical excipients are materials, excluding the API, intentionally included in a drug product to satisfy the criteria for quality by regulatory bodies and safety of patients in terms of manufacturability or composition, performance, and appearance or specifications.[2] According to Moreton,[2] pharmaceutical excipients are a very sundry group of materials which may exist in all the states of matter: liquid, gas, and solid (as well as semi-solid), they can be of natural or synthetic (as well as semi-synthetic) source and of various molecular sizes (simple molecules or very complex polymers).

According to Kolter and Guth,[3] drug delivery systems often require highly functional excipients to achieve the targeted product properties. There are 3 types of excipients based on development approaches: (a) modified excipients (existing physical or purity has been changed)—examples include Kollidon VA 64 Fine, Polyplasdone Ultra, and Tween 80 HP; (b) coprocessed excipients (excipients are formulated to yield new combination)—examples include Aquarius, Ludiflash (Mannitol–Polyvinylacetate–Crospovidone), and StarCap 1500; and (c) novel excipients (chemical entities newly discovered)—examples include Captisol, Kollicoat IR, and Soluplus.

Excipients are used to bring about changes in the pharmacological activity of the drug by altering solubility, dissolution, permeability, and bioavailability.[4] Until recently, excipients were believed to be passive with no biological activity of their own. Changes in the transporter-mediated absorption of substrates and modulations in cytochrome P450 (CYP) enzymes activity have been associated with excipients in several studies.[4-6] Enhancement or inhibition of CYPs activities can change drug metabolism profile and result in either increase in the bioavailability or decrease in the efficacy of the drug.[6] There are more than 55 human CYP homologues, of which 90% of therapeutic drugs are metabolized by CYP1A2,

CYP3A4, CYP3A5, CYP2C9, CYP2C19, CYP2D6, and/or CYP2E1.[7,8] The cytochrome P450 3A (CYP3A) family constitute more than 70% of small intestinal cytochrome P450 and has CYP3A4 as the key enzyme.[9]

Permeability glycoprotein also known as P-glycoprotein (P-gp; *MDR1*; ABCB1) is an efflux transporter which belongs to ATP-binding cassette (ABC) superfamily of transporters, actively transport a wide range of structurally and mechanistically diverse endogenous and xenobiotics across the cell membrane at the energy expense of ATP hydrolysis,[10] and found in blood–brain barrier (BBB), gastrointestinal tract, liver, placenta, and kidneys in humans.[4] P-gp efflux and CYPs activity can profoundly implicate the role of drug pharmacokinetics by clinically altering the administered drug efficacy or resulting to various adverse side-effects due to drug–drug interactions (DDIs), as in the case of multi-administration of drugs and herbal formulations.[10-13] Although DDIs often lead to negative impact on therapy, they can also be leveraged in drug regimens to facilitate the absorption of drugs and increase their bioavailability.[14]

The manufacturing process of many excipients might contain levels of reactive impurities (such as reducing sugar, aldehydes, peroxides, nitrites, organic acids, and metals), which might lead to incompatibilities with APIs in the formulations.[15] Pharmacokinetics/toxicokinetics of excipients is the study of absorption, distribution, metabolism, and excretion (ADME) of excipients in relation to their pharmacological/toxic effects. The chemical structure and chemical and physical properties of the excipients are to be examined first in hazard identification, as these may show possible toxicological issue.[16] In pharmacological study, substrate are compounds actively transported and metabolized by enzymes; inhibitors are compounds that prevent transport or enzymatic activity and increase the level of substrates, whereas modulators or inducers are compounds that interact with another binding site different from where the substrate binds on transporter or enzyme and decrease the level of substrates (increase the bioavailability). Studies have classified different drug compounds interaction with numbers of cytochrome P450 (CYP) isoforms which include imipramine (for 1A2, 2D6, 3A4), omeprazole (for 2C19, 3A), losartan (for 2C9, 3A4), and digoxin (for P-gp).[1,4,17] The need to study the potential pharmacological benefits and toxicological liabilities of commonly used small molecule excipients is therefore becoming increasingly apparent. In this study, the pharmacokinetics and molecular binding of 11 selected common small molecule excipients used in drug formulation were computationally investigated for their effect on the function of P-gp and activity of selected cytochrome P450 isoforms.

Materials and Methods

In silico preparation of ligands

Eleven small molecule excipients were adapted from available published literatures.[4,18,19] Available structure of each of the compounds (ligands) was obtained from the PubChem Compound Database in structure data file (sdf) and canonical SMILES (Simplified Molecular Input Line Entry Specification) format. The ligand structures generated were subjected to 3-dimensional optimizations using ChemSketch and saved as mol2 format. All file conversion to protein data bank (pdb) format were performed using PyMol v2.0.7.

In silico preparation of first-pass targets

Six cytochrome P450 enzymes (CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP2E1) and P-gp, which are key targets involved in first-pass effect of drug metabolism, were selected based on the information available in published literature.[1,4,6,17] The 3-dimensional (3D) structure of each of the cytochrome p450 was obtained from RCSB Protein Data Bank (PDB) database.

Molecular docking studies

The molecular docking studies were performed according to the method of Fatoki et al.[20] Briefly, all water molecules, hetero atoms, and multichain were removed from the crystal structure of the prepared targets using PyMol v2.0.7. The Gasteiger partial charges were added to the ligand atoms prior to docking. The docking parameter of each prepared ligand and each prepared target was setup using AutoDock Tools (ADT) v1.5.6,[21] and molecular docking program AutoDock Vina v1.1.2[22] was employed to perform the docking experiment.

In silico pharmacokinetics and target prediction

The ligands were then subjected to in silico ADME (Absorption, Distribution, Metabolism, and Excretion) screening on SwissADME server.[23] ADME screening was performed at default parameters. In silico prediction of target for the excipients was done using SwissTargetPrediction server where *Homo sapiens* was selected as target organism.[24] The information about medications that contain each of the selected excipients used in this work was obtained from www.drugs.com.

Molecular dynamics simulation

The dynamics of P-gp structure (PDB: 6C0V, chain A) was investigated according to the method of Ugboko et al[25] at pH 7.5. Briefly, from the crystal structure (X-ray structure), PDBFixer implemented in OpenMM v7.3[26] on CPU platform was used to fix the protein. The OpenMM ForceField was instantiated using amber14/protein.ff14SB and amber14/tip3p water model with constraints on the lengths of all bonds involving a hydrogen atom and TIP3P waters were added to a cubic box extending 10 Å beyond the outermost protein atoms with 200 mM NaCl. The energy minimization was conducted until a tolerance of 25 kJ/mol using a Langevin integrator[27]

with a time step of 2.0 fs, temperature of 300.0 K, and collision rate of 5.0 p/s using single precision. Non-bonded forces were modeled using the particle-mesh Ewald (PME) method[28] with a cutoff distance of 10 Å and a Monte Carlo Barostat with pressure of 1 atm with update at interval of 50 steps. The minimized protein was then subjected to fast simulation of structural flexibility using CAB-flex 2.0 server[29] with random number generation seed of 4685 while other parameters were at default settings. The contact map and root-mean square fluctuations (RMSF) of amino acid residues in the server-analyzed protein were obtained.

Results and Discussion

Several studies have been done to determine how to overcome first-pass metabolism of drug by understanding the nature of the substrates, inhibitors, and inducers of these enzymes and transporters as well as the influence of excipients in DDI favorable to bioavailability of API.[4,30] Eleven small molecule excipients used in this study (and their PubChem identification number) were acetyltributyl citrate (6505), ascorbyl palmitate (54680660), aspartame (134601), cetyl alcohol (2682), cetyltrimethyl-ammonium bromide (CTAB; 5974), dibutyl sebacate (7986), docusate sodium (23673837), lactose monohydrate (104938), sodium caprylate (23664772), sucrose stearate (9898327), and tricaprylin (10850). The effect of selected poly-molecule pharmaceutical excipients on cytochrome P450 (CYP) and P-gp has been studied experimentally, of which results showed that most excipients were capable of inhibiting and increasing activity of several different CYP isoforms as well as inhibit P-gp transport function at therapeutic concentration.[4,6] Also, pharmaceutical excipients that are surfactants such as SLS, RH40, Tween 20, and EL35 have been found to attenuate 2 major forms of human carboxylesterase (CES1A and CES2) activities.[31]

The docking parameters and predicted active site amino acid residues are shown in Table 1. Interaction of P-gp with many structurally diverse compounds indicates the presence of multiple binding sites. Two different ligand binding sites were predicted for P-gp in this study, which are possibly for ATP binding (nucleotide-binding domain) and xenobiotic binding (transmembrane domain). The binding free energy between the excipients and selected first-pass metabolic proteins is shown in Table 2. The lesser the binding energy, the more the possibility that the excipient will undergo first-pass effect by the appropriate CYPs and P-gp. At binding free energy cut-off value of −5.0 kcal/mol, the result showed possible modulatory or inhibitory effect by cetyl alcohol on CPY3A4 and P-gp; CTAB on CYP1A2 and P-gp; dibutyl sebacate on CYP2C9, CYP2E1, and P-gp; sodium caprylate on CYP1A2 and CYP3A4; while other excipients in this study have good interaction with the cytochromes and P-gp at value below −5.0 kcal/mol, except interaction of docusate sodium with CYP2C9 and CYP2E1 as well as ascorbyl palmitate with CYP3A4.

Despite efforts on the prediction of P-gp inhibitors or substrates, the accuracy of the prediction models is still a serious challenge.[32] However, the molecular docking results could not differentiate between the excipients that are substrates, inhibitors, or modulators; hence, these compounds are classified as substrates, inhibitors, and modulators in this study. Thus, the results of predicted pharmacokinetics (ADME) as shown in Table 3 provided possible inhibitors of the CYPs and P-gp. Excipients E, G, and H were not substrates for P-gp, whereas others may be inhibitors or modulators of P-gp. Also, excipients C, E, G to J may probably be substrates for CYPs because they were not found as inhibitors for any of cytochrome P450 curated in the SwissADME database.

Hypothetically, small molecule excipient with cumulative binding energy (CBE) which is below −42.5 kcal/mol (such as excipient H) is likely to have high rate of first-pass metabolism and it is termed as CBE-A excipient, whereas small molecule excipient with CBE which is above −42.5 kcal/mol is likely to enter systemic circulation to exert certain level of biological effects, and it is termed as CBE-B excipient (Table 2). Thus, medication that contains 3 or more of CBE-B excipients should be reformulated to yield acceptable bioequivalence such as tramadol hydrochloride and omeprazole (Table 4). Moreover, different brands of medications usually have different excipients composition in their formulation but achieved the same bioequivalence. The incompatibility or interaction of excipients with certain APIs has been reviewed by Bharate et al.[33]

In silico target prediction showed that 3 of the excipients have potential target; CTAB showed 100% and 35% probability of target to Dynamin-1 (UniProt ID: Q05193) and Histamine H3 receptor (UniProt ID: Q9Y5N1), respectively; ascorbyl palmitate showed 65% probability of target to glycogen synthase kinase-3 beta (UniProt ID: P49841), whereas tricaprylin showed 40% probability of target to 5 Protein kinase C (UniProt IDs: P17252, Q02156, Q04759, P24723, and P05129).

Studies on antimalarial activity of CTAB have shown that it interferes with *Plasmodium falciparum* phospholipid metabolism[34,35] and choline kinase[36] as well acts as inhibitor of cyclopropane mycolic acid synthase 1 (Uniprot ID: P9WPB7) in *Mycobacterium tuberculosis*.[37] Also, CTAB is one of the trace components of Aflunov and Foclivia, which are vaccines for influenza virus (Table 4).

This study therefore revealed the effects of first-pass metabolic CYPs and P-gp on the inertness of excipients of pharmaceutical, topical and food applications. Study has also shown that nanoparticles of CTAB increase the intracellular concentrations of P-gp substrates.[38] Previous in vivo study has indicated that ascorbyl palmitate did not inhibit CYP3A4 activity though it was found to be a moderately potent reversible inhibitor of in vitro tested CYP3A4 activity.[39] Thus, the level of inhibition of CYP450 by ascorbyl palmitate as predicted in this study (Table 3) may not indicate significant impact in an in

Table 1. The docking parameters for first-pass-related enzymes and predicted active site amino acid residues.

FIRST-PASS ENZYMES	CENTER GRID BOX (POINTS)	SIZE (POINTS)	SPACING (Å)	PREDICTED BINDING SITE RESIDUES
Cytochrome P450 1A2 (PDB: 2HI4, chain A)	3.511 × 21.453 × 26.116	126 × 126 × 126	0.475	Arg108, Thr124, Trp133, Arg137, Leu144, Ile200, Asp313, Ile314, Ala317, Gly318, Thr321, Val322, Ala325, Phe376, Phe381, Leu382, Thr385, Ile386, His388, Gln411, Leu450, Phe451, Gly452, Met453, Lys455, Arg456, Arg457, Cys458, Ile459, Gly460, Leu463, Ala464, Ile468
Cytochrome P450 3A4 (PDB: 1TQN, chain A)	–19.161 × –23.914 × –13.938	110 × 126 × 126	0.475	Arg105, Ile118, Ser119, Trp126, Arg130, Phe137, Phe302, Ala305, Gly306, Thr309, Thr310, Val313, Leu364, Ile369, Ala370, Leu373, Arg375, Pro434, Phe435, Gly436, Arg440, Asn441, Cys442, Ile443, Gly444, Phe447, Ala448, Met452
Cytochrome P450 2C9 (PDB: 5A5J, chain A)	4.165 × 23.443 × 4.859	126 × 126 × 126	0.475	Trp120, Arg124, Leu131, Ile178, Leu294, Ala297, Gly298, Thr301, Thr302, Thr305, Gln356, Leu361, Ser365, Leu366, His368, Leu391, Pro427, Phe428, Ser429, Ala430, Lys432, Arg433, Ile434, Cys435, Val436, Gly437, Leu440, Ala441, Leu445
Cytochrome P450 2C19 (PDB: 4GQS, chain A)	–79.633 × 21.971 × –42.954	126 × 110 × 126	0.475	Arg97, Ile112, Val113, Trp120, Arg124, Leu131, Ile178, Leu294, Ala297, Gly298, Thr301, Thr302, Thr305, Gln356, Ser365, Leu366, His368, Pro427, Phe428, Ser429, Ala430, Lys432, Arg433, Ile434, Cys435, Val436, Gly437, Glu438, Leu440, Ala441, Glu444
Cytochrome P450 2D6 (PDB: 4WNW, chain A)	–14.240 × –16.021 × 32.595	126 × 126 × 126	0.475	Arg101, Val119, Phe120, Trp128, Arg132, Ile186, Leu302, Ala305, Gly306, Thr309, Thr310, Thr313, Gln364, Val374, His376, Leu399, Pro435, Phe436, Ser437, Ala438, Arg441, Ala442, Cys443, Leu444, Gly445, Leu448, Ala449, Glu452
Cytochrome P450 2E1 (PDB: 3LC4, chain A)	1.146 × –7.303 × 9.089	126 × 126 × 110	0.475	Arg100, Ile114, Ile115, Trp122, Arg126, Leu133, Ile180, Leu296, Ala299, Gly300, Thr303, Thr304, Thr307, Gln358, Leu363, Val364, Asn367, Leu368, His370, Leu393, Pro429, Phe430, Ser431, Thr432, Lys434, Arg435, Val436, Cys437, Ala438, Gly439, Leu442, Ala443, Leu447
P-glycoprotein (PDB:6C0V, chain A)	162.417 × 168.981 × 149.914	80 × 110 × 126	0.875	Asp164, Tyr401, Arg404, Ile409, Asn428, Ser429, Gly430, Cys431, Gly432, Lys433, Ser434, Thr435, Gln475, His587, Arg905, Lys1076, Ser1077, Thr1078, Phe1157, Gln1175, Leu1176, Ser1177, Gly1179, Gln1180
				Ala529, Gln530, Ser532, Gly533, Gly534, Gly535, Tyr1044, Thr1046, Arg1047, Val1052, Ser1071, Gly1073, Cys1074, Gly1073, Cys1074, Gly1075, Lys1076, Ser1077, Thr1078, Gln1118, Gln1201

Table 2. Docking score for the binding free energy between the first-pass metabolic proteins and selected excipients.

FIRST-PASS TARGETS	BINDING ENERGY (KCAL/MOL) OF THE SELECTED EXCIPIENTS										
	A	B	C	D	E	F	G	H	I	J	K
Cytochrome P450 1A2 (PDB: 2HI4)	−5.0	−5.7	−5.5	−4.5	**−7.1**	**−4.0**	−5.1	−6.8	−6.1	−6.0	−4.9
Cytochrome P450 3A4 (PDB: 1TQN)	−5.1	−4.9	−6.6	−5.8	**−3.8**	−4.5	−7.0	**−7.4**	−5.1	−6.1	−5.7
Cytochrome P450 2C9 (PDB: 5A5J)	−5.0	−6.6	−5.3	**−4.2**	−4.4	−5.4	−4.9	**−7.3**	−4.3	−5.1	−5.5
Cytochrome P450 2C19 (PDB: 4GQS)	−5.0	−5.8	−6.1	**−3.9**	−4.2	−4.4	−5.8	**−7.7**	−4.9	−6.3	−5.8
Cytochrome P450 2D6 (PDB: 4WNW)	−5.5	−5.4	−6.5	**−4.0**	−4.4	−4.8	−5.7	**−7.0**	−4.3	−5.4	−5.5
Cytochrome P450 2E1 (PDB: 3LC4)	−5.3	−5.8	−6.2	−4.7	**−4.5**	−6.9	−4.9	**−7.8**	**−4.5**	−5.8	−5.3
P-glycoprotein (PDB:6C0V)	−5.1	−6.9	−6.3	−5.3	−5.7	−5.1	−6.7	**−8.5**	**−4.8**	−6.7	−5.1
Cumulative binding energy	−36.0	−41.1	−42.5	−32.4	−34.1	−35.1	−40.1	−52.5	−34.0	−41.4	−37.8

Selected excipients: (A) acetyltributyl citrate, (B) ascorbyl palmitate, (C) aspartame, (D) cetyl alcohol, (E) cetyltrimethyl-ammonium bromide (CTAB), (F) dibutyl sebacate, (G) docusate sodium, (H) lactose monohydrate, (I) sodium caprylate, (J) sucrose stearate, and (K) tricaprylin (caprylic acid triglyceride). The bold values show the lowest and highest binding energy for the targets (across the row).

vivo pharmacological evaluation. From results of this in silico study which showed that ATBC may not permeate BBB and not act as P-gp substrate, it can be theorized that ATBC may possibly act as inhibitor of P-gp and CYP2C19 in the intestinal cell, with no further systemic distribution to the liver and brain. Investigation of acetyl tributyl citrate (ATBC) in both in vitro and in vivo has shown increase in the CYP3A4 messenger ribonucleic acid (mRNA) level and enzyme activity, both in the human intestinal cells but not in liver cells.[40] The P-gp mediates decrease in the availability of substrates for CYP thereby affecting the apparent metabolic activity.[17] The pharmacokinetics of a drug may be altered when formulated with excipients which inhibit or induce P-gp. The inhibition and induction of P-gp often result in increase and decrease in bioavailability, respectively. The high binding energy of lactose monohydrate implicated it as good substrate for both CYPs and P-gp, thus resulting in lower gastrointestinal absorption and lower bioavailability; this showed that the affinity of ligand to protein target does not often connote inhibitory mode of action.

Tricaprylin is the triester of caprylic acid with glycerin and has applications as food additive, cosmetics agent, and pharmaceutical excipient. Study has shown extensive damage of tumor cells (lymphoma implants in the liver) in rats after oral dosing with tricaprylin.[41] Increase in the level of 7 cytochrome P450 which includes CYP2E1 and CYP1A2 in cerebrum and cerebellum microsomal fractions of rat brain has been found to be associated with excessive amount of aspartame, whereas hepatic microsomal fractions showed no differences in CYPs concentration and activity,[42] but CYP3A2 activity was induced in the brain and liver.[43] This corroborates the result of this study which predicted that aspartame has no inhibitory effect on the hepatic and intestinal CYPs and P-gp, and its inability to cross BBB suggests no induction effect on the brain CYPs and P-gp.

The expression P-gp at endothelial capillaries of the brain aids BBB against xenobiotic access to the central nervous system. As shown in Table 3, it could be deduced and hypothesized that (a) for any small molecule that is BBB permeant and P-gp substrate simultaneously cannot be an inhibitor of CYPs, (b) for any small molecule that is not BBB permeant and P-gp substrate simultaneously or independently will be an inhibitor of 1 or more CYPs. Simultaneous inhibition of the cytochrome or ABC transporter in the mammal host and the target organism has been found to enhance pharmacokinetics and pharmacodynamics of the drug "Ivermectin."[44] Theoretically, the synergistic effect of this simultaneous inhibition of CYP and P-gp has been proposed to increase penetration of the drug into the central nervous system and facilitate interaction with the GABA receptors.[44]

The superimposition of the structure and contact map of 10 models of flexibility simulation of human P-gp is shown in Figures 1 and 2, respectively. Biochemical evidence seems to favor the idea that the 2 nucleotide-binding domains (NBDs) are likely in a "constant contact" mode.[45,46] Eight major regions which have been identified on the human P-gp are 1 to 51 (region 1), 73 to 119 (region 2), 141 to 188 (region 3), 237 to 296 (region 4), 347 to 710 (region 5) containing the NBD1, 778 to 832 (region 6), 885 to 936 (region 7), and 995 to 1280 (region 8) containing the NBD2, and others are non-membrane regions, 210 to 215, 318 to 325, 732 to 756, and 958 to 973.[47] The RMSF in Armstrong (Å) amino acid residues of human P-gp shown in Figure 3 shows that regions 1, 2, 3, and 6 are relatively stable within the range of 0 to 1.0 Å, whereas the fluctuations observed in regions 4, 5, 7, 8, and other non-membrane regions could account for conformational changes which facilitate the open and close motions in transport function of P-gp. Esser et al[48] have found that the open-and-close motion of P-gp is structurally linked to conformational changes of each individual helix of P-gp, and it allows P-gp to change its surface topology within the drug binding pocket. Previous molecular dynamics simulation study of human P-gp has

Table 3. Predicted pharmacokinetics parameters of selected first-pass enzymes.

PARAMETERS	SELECTED SMALL MOLECULE EXCIPIENTS										
	A	B	C	D	E	F	G	H	I	J	K
Molecular weight	402.48	414.53	294.3	242.44	364.45	314.46	444.56	360.31	166.19	608.76	470.68
Molar refractivity	103.43	112.16	73.82	80.19	104.75	91.21	109.51	71.17	40.4	154.81	135.76
Total polar surface area (Å^2)	105.2	113.29	118.72	20.23	0	52.6	118.18	198.76	40.13	195.6	78.9
Consensus LogP	3.47	4.25	−0.13	5.42	2.45	4.79	1.85	−4.06	−0.11	2.84	6.99
ESOL class	Soluble	Moderately soluble	Highly soluble	Moderately soluble	Soluble	Soluble	Moderately soluble	Highly soluble	Soluble	Moderately soluble	Poorly soluble
Gastrointestinal absorption	High	High	High	High	Low	High	Low	Low	High	Low	Low
Blood–brain barrier (BBB) permeant	No	No	No	Yes	Yes	Yes	No	No	Yes	No	No
P-glycoprotein substrate	No	No	No	No	Yes	No	Yes	Yes	Yes	No	No
Cytochrome P450 inhibitor	CYP2C19	CYP2C9, CYP2C19, CYP2D6	–	CYP1A2	–	CYP3A4	–	–	–	–	CYP3A4
Skin permeation Log Kp (cm/s)	−6.42	−4.38	−10.04	−2.73	−6.25	−4.5	−5.36	−11.37	−5.15	−6.66	−2.82
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.17	0.55	0.17	0.55

Selected excipients: (A) acetyltributyl citrate, (B) ascorbyl palmitate, (C) aspartame, (D) cetyl alcohol, (E) cetyltrimethyl-ammonium bromide (CTAB), (F) dibutyl sebacate, (G) docusate sodium, (H) lactose monohydrate, (I) sodium caprylate, (J) sucrose stearate, and (K) tricaprylin (caprylic acid triglyceride).

Table 4. Top medications that contain each of the selected excipients used in this study.

EXCIPIENTS	EXAMPLES OF MEDICATIONS
Acetyltributyl citrate	Budesonide (Enteric Coated), Cardizem CD, Dexmethylphenidate Hydrochloride Extended-Release, **Diltiazem Hydrochloride Extended-Release**, Glipizide Extended Release, Mesalamine Delayed-Release, Metoprolol Succinate Extended-Release, **Potassium Chloride Extended-Release**, etc.
Ascorbyl palmitate	Animi-3 with Vitamin D, Enjuvia synthetic B, Integra Vitamin B Complex with C and Iron, Prenatal Plus Prenatal Vitamin with Iron Fumarate, Tretinoin, Tylenol Regular Strength, Udamin SP dietary supplement, etc.
Aspartame	Aripiprazole (Orally Disintegrating), Cetirizine Hydrochloride (Chewable), **Clonazepam**, Lamotrigine (Orally Disintegrating), Methylphenidate Hydrochloride (Chewable), Mirtazapine (Orally Disintegrating), Olanzapine (Orally Disintegrating), Ondansetron Hydrochloride (Orally Disintegrating), Risperidone (Orally Disintegrating), Rizatriptan Benzoate (Orally Disintegrating), Zolmitriptan (Orally Disintegrating)
Cetyl alcohol	Amoxicillin and Clavulanate Potassium, Budesonide, Dextroamphetamine Sulfate Extended Release, Lansoprazole Delayed Release (Orally Disintegrating), **Methylphenidate Hydrochloride Extended-Release**, **Omeprazole Delayed Release**, Ursodiol, etc.
Dibutyl sebacate	Bupropion Hydrochloride Extended-Release (XL), **Methylphenidate Hydrochloride Extended-Release**, **Omeprazole Delayed Release**, **Potassium Chloride Extended-Release**, Propranolol Hydrochloride Extended-Release, **Tramadol Hydrochloride Extended Release**, Venlafaxine Hydrochloride Extended Release, Verapamil Hydrochloride Extended-Release, etc.
Docusate sodium	Euro-Docusate, Docusate-Humanity, Otitex, Glyoktyl, Vencoll, Humectol-D, etc.
Lactose monohydrate	Acetaminophen and Oxycodone Hydrochloride, Alprazolam, Cetirizine Hydrochloride, **Clonazepam**, Cyclobenzaprine Hydrochloride, Oxycodone Hydrochloride, **Tramadol Hydrochloride**, etc.
Sucrose stearate	ConZip, **Diltiazem Hydrochloride Extended-Release**, Esomeprazole Magnesium Delayed-Release, Prasugrel Hydrochloride, Tiazac, **Tramadol Hydrochloride Extended Release**, etc.
Cetyltrimethyl-ammonium bromide (CTAB)	Aflunov, Foclivia, Xylonor, Savlodil, and other topical antiseptic cetrimide, shampoos, and cosmetics (ref. https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:3567)
Tricaprylin and sodium caprylate	There is no validated scientific data to support the use of caprylic acid and its derivatives as sole medicine for any health problem (ref. https://www.drugs.com/npc/caprylic-acid.html).

Bolded medications contained more than one of the excipients investigated in this study.

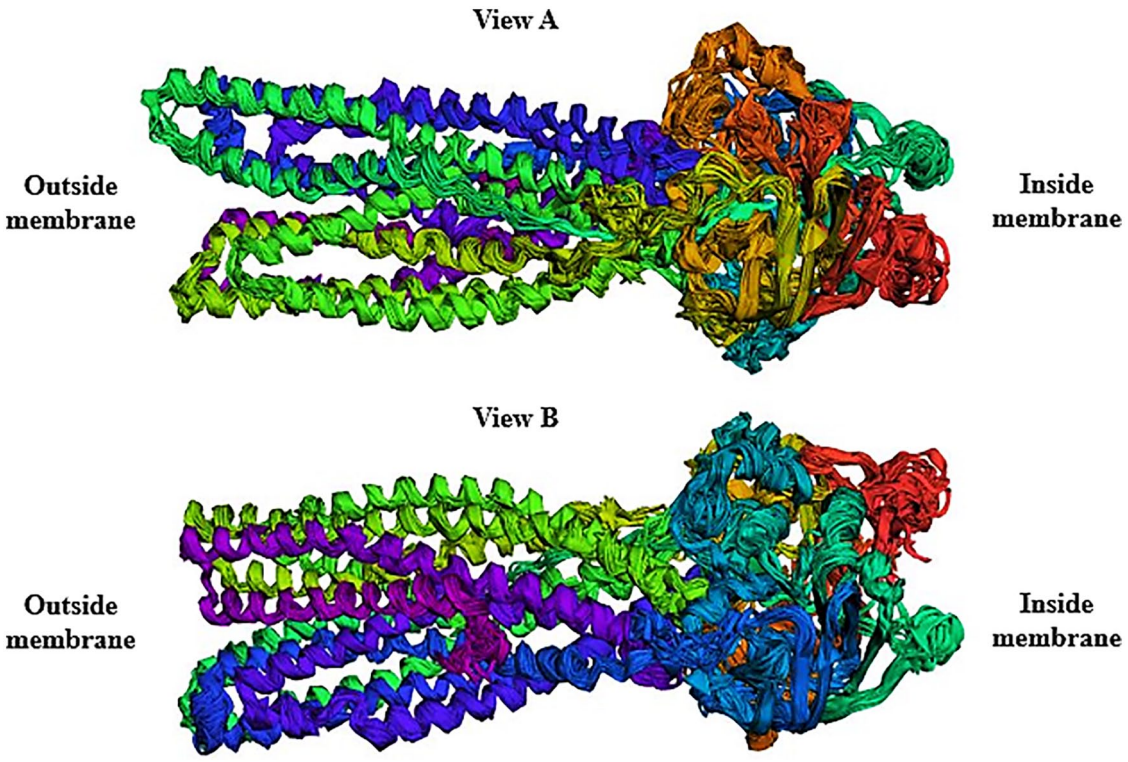

Figure 1. Superimposition of top 10 simulated structures of human P-glycoprotein obtained from CAB-flex 2.0 server.

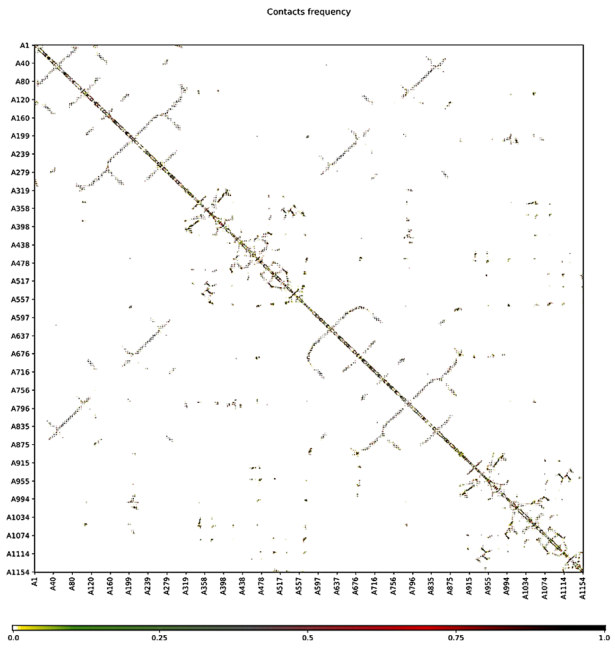

Figure 2. Contact map of superimposition of top 10 simulated structures of human P-glycoprotein obtained from CAB-flex 2.0 server.

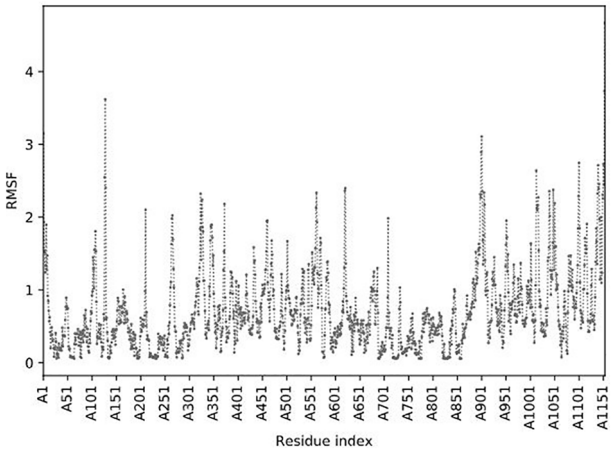

Figure 3. Root-mean square fluctuations (unit in Å) of amino acid residues in the human P-glycoprotein obtained from CAB-flex 2.0 server.

shown that residues D177 and N820 interact with tariquidar when the NBDs were in the closed positions in these simulations, and that this interaction may be essential in its mechanism of inhibition.[49] There is strong evidence that tariquidar has a much higher affinity to P-gp and slower off-rate from P-gp than vinblastine, a good transport substrate.[50] A study has shown small RMSD changes of 1 to 2.5 Å for a series of P-gp transport substrates.[51]

The therapeutic efficacy of API must be balanced by the functionality of the excipients to assure the quality and safety of the drug.[52] The safety-toxicity assessments are classically based on the appearance of gross morphological changes rather than the effects on a cellular level, whereas the ability of excipients in modifying the pharmacological activity by modulating transporters of an active drug which could lead to toxicity should be rational approach of assessment.[53] In Australia, consultation has been made to increase access to ingredient information online to assure therapeutic goods administration to the consumers.[54] Many potential drugs might have been discarded as a result of limited efficacy due to unsuitable excipients. Specific cases of variation in 2 excipients (mannitol or trehalose, and metoprolol or sorbitol), leading to counteraction of the activity of the active agent have been reported.[55,56] Comprehensive review on the potential for excipients to alter the individual response to or tolerance of a medication brand has been published recently by Page and Etherton-Beer,[57] which could affect pediatric and adult populations with different toxicological outcomes.[58,59] Thus, excipients bioavailability, API-excipient, and excipient–excipient interactions should be investigated during drug formulation, as this could be the basis of specific side effects.

Conclusions

This study showed that some excipients can change drug metabolism through the effects on cytochrome P450 activity and P-gp function. The presence of some excipients in drug formulation for oral administration in single or multiple drugs prescription in the presence or absence of gastrointestinal food contents may lead to pharmacokinetic interactions and possibly reduce efficacy on the pathogen or host organism molecular targets, which are relevant to microbial drug resistance and toxicity in pediatric and adult populations. Hence, the clinical significance of findings from this in silico work should be taken into consideration during drug formulation and administration.

Author Contributions

Authors THF, OI, and OAA designed the study, while THF conducted the analyses. All authors were involved in the interpretation of the results, preparation and revision of the manuscript, and approved the final version of the manuscript.

ORCID iDs

Toluwase Hezekiah Fatoki https://orcid.org/0000-0003-3202-9855
Abiodun Samuel Oyedele https://orcid.org/0000-0001-9528-5288

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