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Docking-based preliminary study on the interactions of bile acids with drugs at the transporter level in intestinal bacteria

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Abstract. – OBJECTIVE: The aim of this study was to estimate the binding-affinities of different bile acids towards drug transporters in *Lactobacillus acidophilus* and *Bifidobacterium longum* in order to predict the influence of bile acids and probiotics interactions on drug pharmacokinetics.

MATERIALS AND METHODS: In order to study interactions of bile acids with transporters of intestinal bacteria, molecular-docking step was performed, using SwissDock web-service. For the purpose of comparison, two natural bile acids, cholic acid (CA) and deoxycholic acid (DCA), and one semi-synthetic bile acid, 12-monoketocholic acid (MKC), were studied in parallel. The free-binding energy was used as the main criterion for ranking ligands.

RESULTS: Studied bile acids exhibited different binding affinities towards bacterial transporters with MKC showing the most prominent effect. For the majority of studied transporters, the estimated affinities of bile acids decreased in the following order: MKC–CA–DCA. Namely, 38.7% of examined transport proteins gave the lowest free-binding energy with MKC. The weak inverse relationship between numbers of hydrogen bonds and estimated free-binding energies was revealed.

CONCLUSIONS: The predominant effect of MKC for the majority of studied transport proteins suggests that keto group at carbon 12 of the steroid core has a significant influence on the properties of MKC and consequently, on interactions with membrane transporters. Present findings might have a role in the prediction of potential influence of bile acids and probiotics on drug pharmacokinetics.

Key Words:
Docking study, Probiotics, Transport proteins, Bile acids, Drug transport.

Introduction

Peroral administration of drugs is by far the most favorable route for drug delivery considering the issues of safety and convenience in administration. For orally administered drugs, good bioavailability and low intra- and inter-patient variability are desirable characteristics. It is well known that poor drug bioavailability is one of the major causes of therapeutic variability, associated with the variable drug exposure[1].

Intestinal microflora provides a rich source of inter- and intra- individual differences in drug metabolism[2]. Namely, gut microflora can be regarded as a complex ecosystem composed of 10^{13} to 10^{14} microorganisms with huge metabolic capacity, qualitatively and quantitatively different from the body cells and organs[3,4]. There is a substantial body of evidence that microbiomes vary between populations and individuals with measurable consequences on pharmacokinetic properties of drugs[5]. Each individual organism is thought to develop a unique and complex intestinal flora profile (i.e. "bacterial fingerprint"), which is affected by a number of factors including genetic background, diet, medications, disease and environmental exposure[6].

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Therefore, gut microflora is considered to exert more pronounced effect on drug metabolism than originally thought, thus becoming the focus of intensive research in the field of personalized medicine. Poorly soluble and/or poorly permeable drugs that are incompletely absorbed from gastrointestinal tract are particularly good candidates to be exposed to gut microflora[7].

The specific physico-chemical and biological properties of bile acids have enabled their application in the development of drugs as a promising pharmaceutical tools for the delivery of active agents[8,9]. Bile acids, as endogenous amphipathic steroid compounds, have a unique ability to modulate the drug transport across various biological membranes being the topic of intensive research[10-13]. Despite the similar chemical structures, bile acids exhibit diverse physical properties and even more divergent biological characteristics[14]. The effect of bile acids on drug penetration had been thought, for a long time, to occur just due to detergent properties causing the opening of tight junctions. Recently, it has been found that bile acids may also modulate transcellular permeation exhibiting effects at the level of membrane transporters[8,13,15].

Cell membrane transporters have a high physiological relevance in drug pharmacokinetics and represent over a third of all drug targets[16]. Although the substrates for transport proteins have very different structures, they share similar physical characteristics, such as high hydrophobicity, an amphiphilic nature, and a positive or neutral charge[17].

The effect of bile acids on drug transporters is an actual challenging topic due to consequent influence on the efficacy and safety profiles of drugs[18]. Research on this subject has been mostly restricted to the impact of bile acids on the eukaryotic drug transporters, which was confirmed by several studies[10,15,19,20]. However, the majority of prokaryotic transporters has close sequence homologues in eukaryotes and possesses the similar specificity for substrates[21]. To date, no studies on the potential interactions of bile acids with multidrug transporters in intestinal bacteria have been performed. Therefore, the main purpose of this work was to assess the affinities of different bile acid species towards bacterial multidrug transporters and to propose the potential mechanism of their influence on drug transport across bacterial membrane.

The effective way to predict binding affinity of ligands to proteins *in silico* is by docking simulation that is used in this study. As a representative of gut microflora, we chose *Lactobacillus acidophilus* NCFM (LA) and *Bifidobacterium longum* NCC2705 (BL), as the most commonly commercially used probiotic bacterial strains and also abundant part of gut microflora. An investigation of their interactions with bile acids at molecular level could provide valuable information on understanding drugs pharmacokinetics and might also influence future studies on the drug metabolism by gut microflora. Furthermore, better characterization of other factors within intestinal microenvironment, such as bile acids, that influence drugs bioavailability, might result in improved pharmacokinetic optimization in discovery and development settings.

Materials and Methods

Docking study was carried out to compare the binding affinities of three different bile acids: CA, MKC and DCA to drug membrane transporters in LA and BL in order to reveal which of them has the highest affinity to bacterial transport proteins and, therefore, the highest possibility for interactions with drug molecules.

Transporters

The list of membrane transporters for LA and BL was obtained from relational database – *TransportDB*, available at http://www.membranetransport.org/, where the complete lists of the transporters from each organism can be accessed. For individual transporter types, a detailed list of transporters with their possible substrates is shown. Considering the potential substrates, we focused on drug transporters due to their implication in drug metabolism and final therapeutic response.

The amino acid sequences of all studied transport proteins were obtained from NCBI database in FASTA format to be utilized for the prediction of their 3D structures. The 3D structures of proteins were predicted by I-TASSER server (Iterative Threading ASSEmbly Refinement), which applies homology modeling and *ab initio* tools for the prediction of the 3D structure of a protein. The obtained 3D structures are given a confidence score (c-score) which is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range from -5 to 2, where a higher value indicates a model with a more reliable

structure and vice-versa. In general, models with c-score > -1.5 are expected to have a correct fold [22]. Proteins with c-score below -1.5 were not taken into further consideration and were not analyzed in docking studies.

Ligands

3D structures of CA and DCA in mol2 format were obtained from ZINC database[23]. Structure of MKC which is not available in Zinc database was drawn and optimized in ACD/ChemSketch, a freeware from Advanced Chemistry Development (ACD Labs, Toronto, ON, Canada) for chemical structure drawing and subsequent optimization.

Docking Study

Molecular docking is the search for the energetically favorable binding pose of a ligand to a macromolecular receptor. The aim of the docking program is to find optimal ligand/protein configuration and accurately (at least consistently) predict the free-binding energy of the complex.

Docking step was performed using molecular docking program SwissDock web service which uses calculations performed in the CHARMM force field with EADock DSS[24]. The required input format of the receptor is PDB (Protein Data Bank) and of ligand is MOL2. The ligand and receptor files in the appropriate formats were uploaded to the web-based server for docking study. The docking was performed using the 'Accurate' parameter at otherwise default parameters, with no region of interest defined (blind docking approach) since the exact binding sites for transport proteins of bacteria have not been conclusively proven yet. Results were downloaded and visualized in UCSF Chimera package[25]. Simultaneously, their CHARMM energies were estimated on the grid[26].

Many criteria from docking results can be used for estimating binding affinity including, free-binding energy (ΔG), full-fitness score, hydrogen binding and total free energy[27]. In our study, we chose the free-binding energy as the main criterion for ranking the most powerful ligands. The lower estimated free energy of binding indicates the higher binding affinity.

The interacting interface was scanned for residues conferring the binding topology. Pearson's coefficients were calculated to examine the degree of correlation between the number of hydrogen bonds and free-binding energies.

Results

Transporters

The number and classification of all membrane transporters for LA and BL obtained from *TransportDB* are presented in Table I.

Each transporter is classified into the ATP-binding cassette (ABC) superfamily or secondary transporters. Contrary to the eukaryotic cells where multidrug transporters mostly belong to the ABC family, the majority of bacterial multidrug transporters characterized up to now, belong to the group of secondary transporters[17].

The total number of membrane transport proteins with drug as a substrate was 20 for BL (6 ABC and 14 secondary transporters) and 30 for LA (14 ABC and 16 secondary transporters). All selected drug transport proteins with group they belonged, possible substrate, c-score given by I-Tasser are listed in Table II.

The majority of predicted 3D structures for 50 examined transport proteins, using I-Tasser, had a high confidence score reflecting the reliability of the obtained structures. Proteins with c-score below -1.5 were not taken into further considera-

Table I. Number and classification of membrane transporters for *Lactobacillus (L.) acidophilus* NCFM and *Bifidobacterium longum* NCC2705.

Transporter type	*L. acidophilus* NCFM		*B. longum* NCC2705	
	Number	Percent	Number	Percent
ATP-dependent	61	36.1%	62	44%
Ion channels 7	4.1%	7	5%	
Phosphotransferase system	23	13.6%	3	2.1%
Secondary transporter	77	45.6%	68	48.2%
Total transporter proteins	169	100%	141	100%

Table II. All selected drug transport proteins from and *Bifidobacterium longum* NCC2705 (a) and *Lactobacillus acidophilus* NCFM (b) with group they belonged, possible substrate, c-score given by I-Tasser, and selection for further docking analysis.

Transporter proteins	Type	Possible substrate	C-score I-TASSER	Docking study
(a)				
BL0162	ATP-dependent	Multidrug	0.45	✓
BL0163	ATP-dependent	Multidrug	1.09	✓
BL0179	ATP-dependent	Multidrug	0.29	✓
BL0180	ATP-dependent	Multidrug	1.15	✓
BL1766	ATP-dependent	Multidrug	-0.70	✓
BL1767	ATP-dependent	Multidrug	-0.17	✓
BL0575	Secondary DMT	Drug/metabolite	-3.04	×
BL1406	Secondary DMT	Drug/metabolite	-1.59	×
BL1444	Secondary DMT	Drug/metabolite	-2.02	×
BL1566	Secondary DMT	Drug/metabolite	-1.39	✓
BL0037	Secondary MFS	Multidrug efflux	-0.74	✓
BL0251	Secondary MFS	Multidrug efflux	-0.9	✓
BL0332	Secondary MFS	Multidrug efflux	-0.46	✓
BL0681	Secondary MFS	Multidrug efflux	-0.84	✓
BL0919	Secondary MFS	Multidrug efflux	-1.19	✓
BL0920	Secondary MFS	Multidrug efflux	-1.83	×
BL1699	Secondary MFS	Multidrug efflux	-0.59	✓
BL1703	Secondary MFS	Multidrug efflux	0.14	✓
BL0432	Secondary MOP Flippase	Multidrug efflux	-1.50	×
BL1082	Secondary MOP Flippase	Multidrug efflux	1.22	✓
(b)				
LBA0246	ATP-dependent	Multidrug	-0.39	✓
LBA0247	ATP-dependent	Multidrug	-2.43	×
LBA0574	ATP-dependent	Multidrug	1.9	✓
LBA0575	ATP-dependent	Multidrug	1.46	✓
LBA0594	ATP-dependent	Multidrug	0.86	✓
LBA0597	ATP-dependent	Multidrug	0.98	✓
LBA1188	ATP-dependent	Multidrug	0.93	✓
LBA1276	ATP-dependent	Multidrug	1.52	✓
LBA1277	ATP-dependent	Multidrug	1.55	✓
LBA1657	ATP-dependent	Multidrug	1.11	✓
LBA1821	ATP-dependent	Multidrug	0.77	✓
LBA1822	ATP-dependent	Multidrug	1.93	✓
LBA1876	ATP-dependent	Multidrug	-0.14	✓
LBA1933	ATP-dependent	Multidrug	1.26	✓
LBA1884	Secondary DMT	Drug/metabolite	-2.68	×
LBA1885	Secondary DMT	Drug/metabolite	-2.54	×
LBA1887	Secondary DMT	Drug/metabolite	-1.67	×
LBA0164	Secondary MFS	Multidrug efflux	0.37	✓
LBA0251	Secondary MFS	Multidrug efflux	0.81	✓
LBA0252	Secondary MFS	Multidrug efflux	-1.32	✓
LBA0552	Secondary MFS	Multidrug efflux	-1.05	✓
LBA0566	Secondary MFS	Multidrug efflux	-1.20	✓
LBA0567	Secondary MFS	Multidrug efflux	1.15	✓
LBA0753	Secondary MFS	Multidrug efflux	-0.38	✓
LBA1429	Secondary MFS	Multidrug efflux	0.90	✓
LBA1446	Secondary MFS	Multidrug efflux	-0.58	✓
LBA1471	Secondary MFS	Multidrug efflux	0.62	✓
LBA1621	Secondary MFS	Multidrug efflux	-0.97	✓
LBA1853	Secondary MFS	Multidrug efflux	0.49	✓
LBA1854	Secondary MFS	Multidrug efflux	-0.91	✓

tion. Accordingly, 5 transport proteins for BL and 4 proteins for LA did not fulfill these criteria and were not further analyzed in docking studies. In other words, 26 transporters of LA and 15 of BL were included in docking studies.

Ligands

For the purpose of comparison, two natural bile salts, CA and DCA, and one semisynthetic bile salt, MKC, were studied in parallel. 3D structures of selected bile acids in mol2 format are presented in Figure 1.

Docking Study

Docking results expressed as ΔG (kcal/mol) obtained by SwissDock software are shown in Figure 2. The attempt to perform docking studies failed for one transporter (BL1766) from overall 41 analyzed, due to complexity of the ligand/protein structure for the docking program.

Namely, from all 40 examined transport proteins, 19 (47.5%) gave the lowest binding energy in combination with MKC. For BL, the most prominent effect of MKC was observed in the cases of ABC transporter – BL1767 and secondary transporter – BL1566. On the other hand, in LA the greatest differences were for LBA1821 from ABC family and LBA0753 from the family of secondary transporters.

Analyzing docking results, the second ranked bile acid was CA that gave the lowest docking result with 13 of 40 (32.5%) transporters. The most notable differences in docking score, regarding the CA, were estimated for BL0681 (secondary transporter in BL), and LBA0575 (ABC transporter in LBA).

For only 8 transporters (20%), DCA was estimated to have the highest affinity compared to other examined bile acids. This is the most expressed in the cases of BL0179 (ABC transporter) and LBA0552 (secondary transporter).

Transporter-Bile Acid Complexes

Figure 3 represents the visualization of the most energetically favorable binding of the CA (A), MKC (B) and DCA (C) into the protein BL1767. Hydrogen bond interactions between the selected transport protein with all studied bile acids, within the range of 3 Å distance, visualized by Chimera are also shown.

Complex of DCA with BL1767 shows 3 hydrogen bonds and all of them originates from Arg 10, with distances of 2.076, 2.276 and 2.076 Å. Arg 10 interacts with MKC by 2 hydrogen bond interactions with 2.092 Å and 2.702 Å distances, respectively. Besides, there is one hydrogen bond interaction between MKC and Thr 149 with 2.385 Å distance. On the other hand, the most energetically favorable binding mode of CA into the selected protein shows no hydrogen bonds interactions.

The correlation between hydrogen bond interactions and estimated free-binding energies for all studied transport proteins revealed weak inverse relationship between the variables (Pearson's coefficient was -0.33). Based on the calculated Pearson's coefficient, it may be concluded that, in addition to the hydrogen bond interactions, the hydrophobic interactions also contribute to the stability of analyzed complexes.

Discussion

Given that bile acids could affect the transport of multiple drugs by direct action on drug transporters, the objective of this study was to use computational servers and softwares to estimate the binding affinities of three different bile acids towards transport proteins in LA and BL.

The molecular docking results presented in this study revealed that studied bile acids exhibit differ-

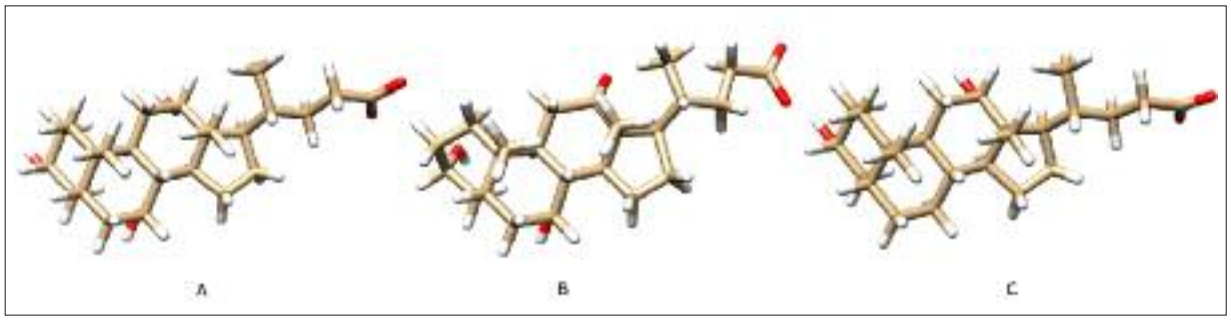

Figure 1. Structures of selected bile acids in mol 2 format ***(A)*** cholic acid ***(B)*** 12-monoketocholic acid ***(C)*** deoxycholic acid.

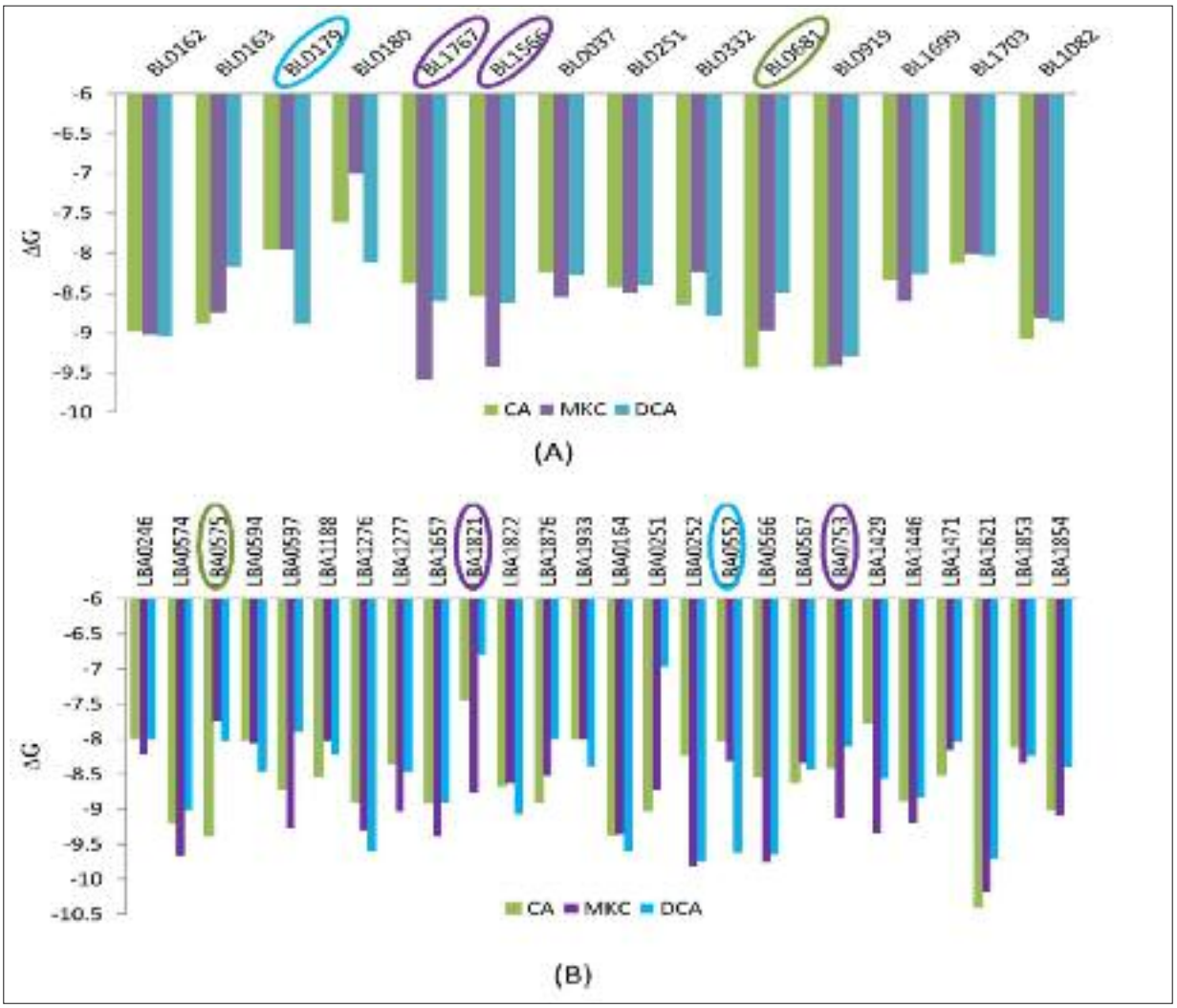

Figure 2. Results of docking studies for drug transporters in *Bifidobacterium longum* ***(A)*** and *Lactobacillus acidophilus* ***(B)*** with bile acids (CA, MKC, DCA) expressed as free-binding energies (ΔG).

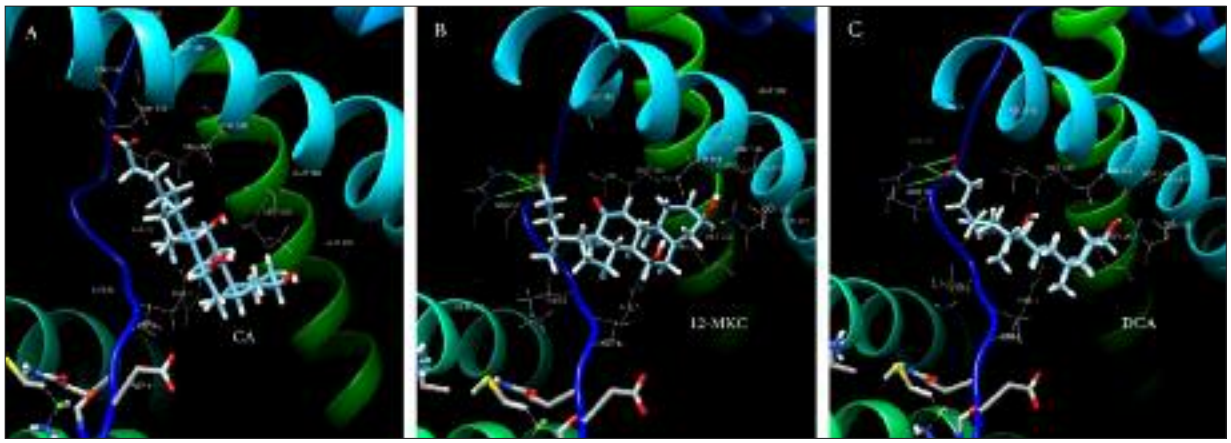

Figure 3. The visualization of the most energetically favorable binding of the CA ***(A)***, 12-MKC ***(B)*** and DCA ***(C)*** into the BL1767. Hydrogen bond interactions between selected transport proteins with all selected bile acids, visualized by Chimera are also shown.

ent binding affinities towards bacterial transporters. The lowest binding energy for the majority of examined transporters was estimated for MKC. Analyzing docking results, the second ranked bile acid was CA, while DCA gave the lowest binding energies with the lowest number of proteins.

Different affinities of studied bile acids towards bacterial transporters may be explained by differences in their structures. Namely, CA and MKC have similar structures, both with hydroxyl group at position 7, differing only in hydroxyl or a keto group at carbon 12 of the steroid core. Comparing to CA, DCA is a dihydroxy bile salt with one hydroxyl group less at the position 7 and consequently with less hydrophilic properties[28]. The hydroxyl groups, oriented towards the α-side of the steroid nucleus, and the carboxylic side chain afford them the more hydrophilic character. On the contrary, the hydrophobic methyl groups (at C-18 and C-19) are oriented towards the β-side[14]. The most prominent effect of MKC for the majority of studied transport proteins suggests that keto group at carbon 12 of the steroid core has a significant influence on the properties of MKC and consequently, on interactions with membrane transporters. However, further *in vitro* and *in vivo* experimental validation is highly recommended to confirm the accuracy of these computationally obtained results.

Although there are still no studies on bile acids interactions with prokaryotic transporters, the obtained results are in accordance with previous findings related to some eukaryotic transporters. Namely, examining the effect of bile salts on transcellular transport of rhodamine 123 across the blood brain barrier, Yang et al[19] showed that, of four examined bile salts [CA, DC, MKC and taurocholate (TC)], MKC produced the greatest increase in uptake and significant reduce of rhodamine 123 efflux, probably by inhibition of P□glycoprotein (P-gp). Al-Salami et al[13] found that MKC induced reduction of mucosal to serosal permeation of gliclazide in healthy rats, which may be the result of the selective inhibition of multidrug resistance protein 3 (MRP3). Furthermore, the *in vitro* experiments have shown that P-gp function was not affected by CA, DC and TC, whereas MKC, taurolithocholate (TLCA), taurochenodeoxycholate (TCDC), glycochenodeoxycholate (GCDC) and ursodeoxycholate (UDC) inhibit P-gp mediated drug transport[15]. The absence of a hydroxyl group at position 12 is the only chemical characteristic that is common for TLCA, TCDC, GCDC, UDC and MKC, indicating this feature as a decisive structural property for bile salts to interact with P-gp. Besides, Kim et al[29] suggested that the improved oral bioavailability of lovastatin in rats upon addition of bile salts may be the result of P-gp inhibition.

Conclusions

The molecular docking results presented in this study revealed that studied bile acids exhibit different binding affinities towards bacterial multidrug transporters. The most prominent effect of MKC for the majority of studied transport proteins suggests that keto group at carbon 12 of the steroid core has a significant influence on the properties of MKC and consequently, on the interactions with membrane transporters. The binding of bile acids to multidrug transporters in intestinal bacteria may lead to interactions with drugs, affecting their pharmacokinetic profiles. These findings might have a role in prediction of potential influence of bile acids and probiotics on drug pharmacokinetics. Computational techniques are expected to continue making significant contribution to investigation of membrane transporters and their implication in drug metabolism.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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