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Research Article

SCREENING OF PROTON PUMP INHIBITOR ANTAGONIST FOR HUMAN FOLATE TRANSPORTER

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ABSTRACT

Folate transport is crucial for proper proliferation of cells. Amongst humans, the folate is procured from external sources since it is not synthesized in the human body. Hence specific transporters are involved in uptake of folate. For the malignant cancerous cells, these folate are crucially required on very frequent rate to fulfil the rapid proliferation of the cancerous cells. In the present study, we have comparatively studied three antagonist drug molecules which could be a potential candidate to inhibit the folate uptake by the human Proton Coupled folate transporter (hPCFT). These candidate antagonist include Pemetrexed, 2-[(4-Aminobenzoyl)amino] pentanedioate-pABA-Glu and Methotrexate. Out of these three antagonist drug, the Pemetrexed molecule was observed to specifically bind to the key active site loop ($G^{155}XXG^{158}$) of the hPCFT transporter. The Binding energy of the Pemetrexed was also comparatively lowest (-7.6 kcal/mol). Hence from the present study we conclude that the Pemetrexed antagonist drug is more efficient to inhibit folate transport as compared to the 2-[(4-Aminobenzoyl)amino]pentanedioate-pABA-Glu and Methotrexate antagonist drug molecules.

Keywords: Proton Coupled folate transporter, hPCFT, Proton Pump Inhibitors, Drug screening, Antagonist.

1. INTRODUCTION

Folic acid (or folate) is one of the critical B group vitamins. It is a generic term which includes tetra-hydrofolate (THF) and its subsidiaries. It is a part of polyglutamate family having key role in carbon metabolism system that is in biosynthesis of purine, pyrimidine, formylmethionyl-tRNA, vitamin B5 and some amino acids like methionine, glycine, serine [1-3]. Folic acid is a synthetic and metabolic form of folates with difference in level of oxidation, carbon substitution and extent of polyglutamation. Human beings lack key enzymes of synthesis pathway of folates thus require them in diet [4]. In addition to the role as a constituent of protein, the folate play major role in synthesis of S-adenosyl methionine necessary for methylation of RNA, DNA, neurotransmitters, phospholipid and proteins like histones. Thus lack of synthesis and transportation of folate leads to folate deficiency in tissues like bone marrow, central nervous system and gastro intestinal tract. Absence of folate synthesising proteins in higher organisms salvage the exogenous uptake of folates which implies the existence of different types of folate transporters [5]. Since, folates are hydrophilic molecules hence do not cross biological membrane by diffusion process. In context with this, mammalian cells have adapted a transport system for cellular uptake of folate cofactors through transmembrane folate transporters [6].

The major facilitative folate transporters are: (i) Reduced Folate Carrier (RFC) which is anionic exchanger responsible to deliver folate from blood plasma to peripheral tissues (ii) Human Proton Coupled folate transporter (hPCFT) with transportation pattern other than RFC specialised in malignant cells and (iii) folate receptors (FR α and β) work by endocytosis in renal cells and macrophages (7-11]. In the present study screening antagonist against the Proton Coupled folate transporter (PCFT) involved in malignant cells was performed. Inhibition of PCFT is supposed to inhibit the proliferation of cancerous cells and hence will provide possible drug candidates against cancer [12-14]. The present study compares the inhibitory mechanism of three prominent proton pump inhibitory drugs viz. Pemetrexed, 2-[(4-Aminobenzoyl) amino] pentanedioate - pABA - Glu and Methotrexate.

The Human proton-coupled folate transporter (hPCFT) has recently been found to be inhibited another proton

pump inhibitor "myricetin" (3_5_7_Trihydroxy_2_(3_4_5_trihydroxyphenyl)_4H_chromen_4_one) [15, 16]. At the same time the mutant G158N-substituted hPCFT was found to be transformed to be insensitive to myricetin. Hence the G158 residue of the hPCFT possibly plays an important role in folate transport. In our study we have also considered the flexible loop ($G^{155}XXG^{158}$) in which the G158 is a conserved residue and acts as target for the chosen antagonist drug molecules.

2. MATERIAL AND METHODS

2.1. Retrieval of three dimensional structure of candidate antagonist.

The three dimensional structure of the candidate antagonist drugs were retrieved from the Pub Chem library. All the three drugs carries identifiers as: 2-[(4-Aminobenzoyl)amino]pentanedioate-pABA-Glu (Conformer3D_CID_5103842); Pemetrexed (Conformer3D_CID_135410875); and Methotrexate (Conformer3D_CID_126941).

2.2. Retrieval of protein sequence of hPCFT

The protein sequence of the hPCFT was retrieved from NCBI. The accession id of the retrieved hPCFT protein sequence was Uni Prot KB/Swiss-Prot: Q96NT5.1

2.3. Modelling of hPCFT

Three dimensional protein model of the hPCFT was performed by I-TASSER. The protein sequence of the hPCFT was used as input. The best model out of all the output models with highest C-Score of -0.70 was chosen for further study [17-19]

2.4. Molecular interaction analysis of candidate antagonist with hPCFT.

Molecular interaction analysis of all the three drug molecules and the hPCFT model was performed by molecular docking experiment. Protein-ligand based molecular docking was performed by the tool Auto Dock Vina. The algorithm carries out the rigid protein-ligand docking approach [20, 21].

3. RESULTS AND DISCUSSION

Folate transporters are essential to uptake the folate for cellular proliferation. Different classes of transporters are involved in this uptake by different cell lines. The RFC, FR α & β, and hPCFT are the major type of folate transporters involved in different cell line of human. Amongst the cancerous cells the hPCFT transporters play crucial rote to uptake folate and hence are essential for survival of the cancerous cells [8-11, 15, 16, 22-26]. This provides an opportunity to design an antagonist drug to inhibit the folate transport and hence diminish the cancerous cell proliferation.

The present study reveals that the chosen drugs viz. 2-[(4-Aminobenzoyl)amino]pentanedioate-pABA-Glu, Pemetrexed and Methotrexate vary closely bind within the cavity of the hPCFT trans membrane transporter (Figure 1-3).

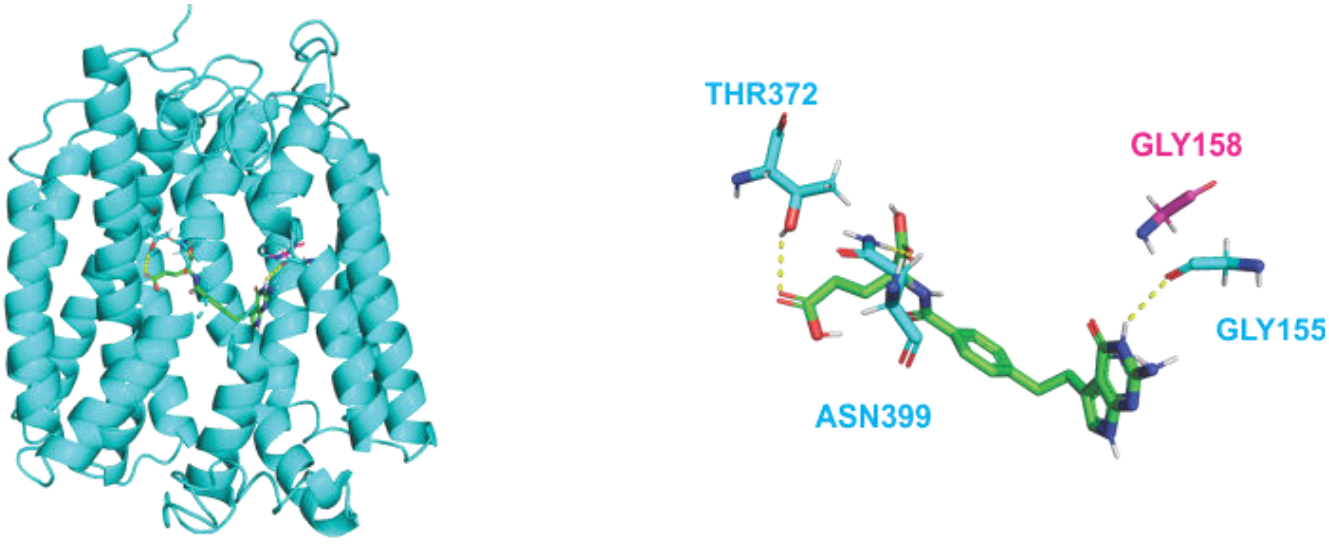

Fig. 1: Molecular interaction of the Pemetrexed antagonist drug molecule within the cavity of the hPCFT transmembrane transporter

Although all three antagonist bind within the hPCFT cavity the drug molecule Pemetrexed shows to bind the same loop ($G^{155}XXG^{158}$) in which the G158 residues is present. Hence the drug molecule Pemetrexed would be more efficient in blocking the binding of folate to the hPCFT transporter as compared to the antagonist 2-[(4-Aminobenzoyl)amino] pentane-dioate-pABA-Glu and Methotrexate. Moreover the binding energy of the Pemetrexed antagonist drug molecule (-7.6 kcal/mol) is lowest as compared to the binding energy of the antagonist 2-[(4-Aminobenzoyl) amino]pentanedioate-pABA-Glu (-6.8 kcal/mol) and Methotrexate (-6.7 kcal/mol) (table 1). The Pemetrexed shows favorable binding with the residues G155 of the active site loop ($G^{155}XXG^{158}$) of hPCFT cavity. Furthermore, amongst all the three antagonist drug molecule the Pemetrexed antagonist drug molecule is the only one which binds to the loop ($G^{155}XXG^{158}$) containing the G158 residue (Figure 1). Hence the drug molecule Pemetrexed could be considered as better inhibitory drug molecule antagonist against the hPCFT transporter to inhibit the folate transport in the malignant cancerous cells. Overall from the present study we propose the Pemetrexed antagonist drug molecule as the potential drug molecule to inhibit the folate transport from the hPCFT transporter implying the inhibition of the proliferation of malignant cancerous cells.

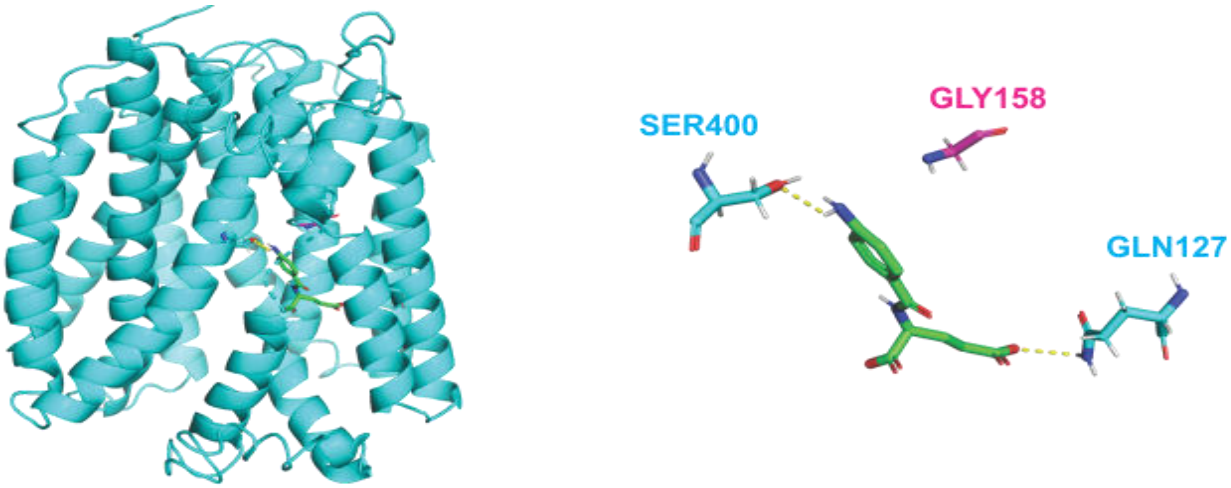

Fig. 2: Molecular interaction of the antagonist 2-[(4-Aminobenzoyl)amino]pentanedioate-pABA-Glu within the cavity of the hPCFT transmembrane transporter

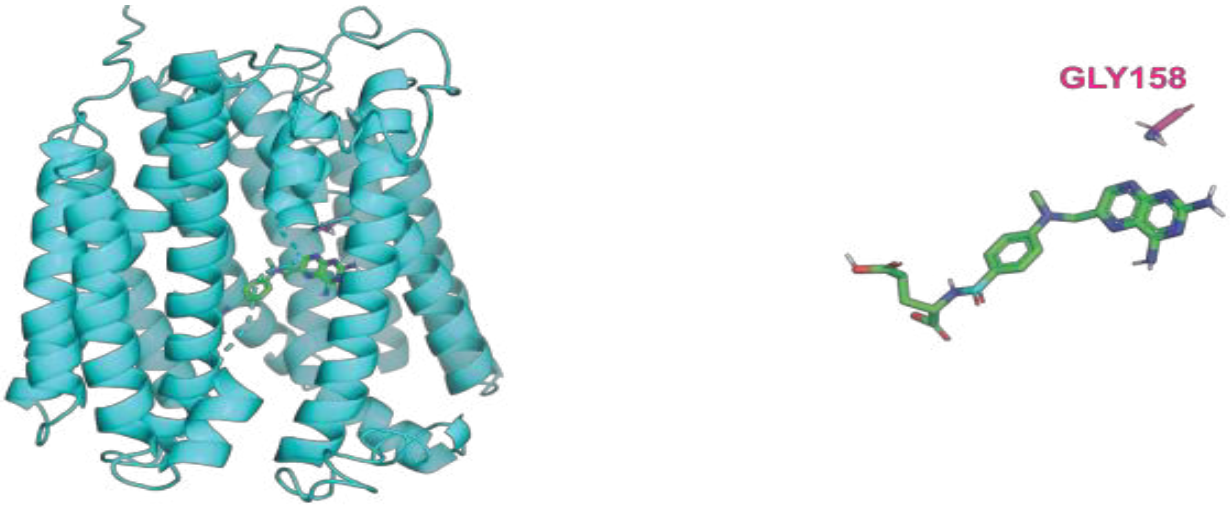

Fig. 3: Molecular interaction of the Methotrexate antagonist drug molecule within the cavity of the hPCFT transmembrane transporter

Table 1: Molecular interaction analysis of the antagonist Pemetrexed; 2-[(4-Aminobenzoyl)amino] pentanedioate-pABA-Glu; and Methotrexate within the cavity of of the hPCFT transmembrane transporter.

Drug molecules	Pub Chem Reference	Binding energy	Binding residues
Pemetrexed	Conformer3D_CID_135410875	-7.6 kcal/mol	Gly155, Thr372, Asn399
2-[(4-Aminobenzoyl)amino] pentanedioate-pABA-Glu	Conformer3D_CID_5103842	-6.8 kcal/mol	Ser400, Gln127
Methotrexate	Conformer3D_CID_126941	-6.7kcal/mol	None

4. CONCLUSION

In this study, we have identified the potential antagonist drug inhibitor against the hPCFT transmembrane folate transporter. The antagonist Pemetrexed have shown to bind G155 residues of the active site loop ($G^{155}XXG^{158}$) which also contains the key G158 residue involved in folate transport, hence the drug Pemetrexed could be considered and tried on animal models as a potential drug inhibitor of the hPCFT transporter. As compared to the other potential drugs viz. 2-[(4-Aminobenzoyl) amino] pentanedioate-pABA-Glu and Methotrexate, the Pemetrexed drug molecule shows favorable binding within the cavity of hPCFT transporter ad hence we conclude the Pemetrexed as a potential drug antagonist against the hPCFT transmembrane folate transporter.

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Conflict of interest

Authors declare that there is no conflict of interest.

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6. REFERENCES

1. Cossins EA, Chen L. *Phytochemistry,* 1997; **45:**437-452.
2. Appling DR. *The FASEB journal,* 1991; **5:**2645-2651.
3. Scott J, Rébeillé F, Fletcher J. *Journal of the Science of Food and Agriculture*, 2000; **80:**795-824.
4. Shane B. *Vitamins & Hormones*, 1989; **45:**263-335.
5. Bekaert S, Storozhenko S, Mehrshahi P, Bennett MJ, Lambert W, Gregory III JF et al. *Trends in plant science*, 2008; **13:**28-35.
6. Matherly LH, Goldman DL. *Vitamins and hormone,* 2003; **66:**405-57.
7. Yamashiro T, Yasujima T, Ohta K, Inoue K, Yuasa H. *Scientific reports*, 2019; **9:**1-0.
8. Takahashi M, Kishimoto H, Shirasaka Y, Inoue K. *Drug metabolism and pharmacokinetics,* 2020; **35:**281-7.
9. Urquhart BL, Gregor JC, Chande N, Knauer MJ, Tirona RG, Kim RB. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 2010; **298:**248-54.
10. Nakai Y, Inoue K, Abe N, Hatakeyama M, Ohta KY, Otagiri M et al. *Journal of Pharmacology and experimental Therapeutics,* 2007; **322:**469-476.
11. Desmoulin SK, Hou Z, Gangjee A, Matherly LH. *Cancer biology &therapy,* 2012; **13:**1355-1373.
12. Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E et al. *Cell,* 2006; **127:**917-928.
13. Qiu A, Min SH, Jansen M, Malhotra U, Tsai E, Cabelof DC, et al. *American journal of physiology-cell physiology,* 2007; **293:**1669-1678.
14. Atabay B, Turker M, Ozer EA, Mahadeo K, Diop-Bove N, Goldman ID. *Pediatric hematology and oncology*, 2010; **27:**614-619.
15. Yamashiro T, Yasujima T, Ohta K, Inoue K, Yuasa H. *Drug metabolism and pharmacokinetics*, 2017; **32:**311-314.
16. Wilson MR, Kugel S, Huang J, Wilson LJ, Wloszczynski PA, Ye J, Matherly LH et al. *Biochemical Journal,* 2015; **469:**33-44.
17. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. *Nature methods,* 2015; **12:**7-8.
18. Roy A, Kucukural A, Zhang Y. *Nature protocols*, 2010; **5:**725-738.
19. Zhang Y. *BMC bioinformatics,* 2008; **9:**1-8.
20. Trott O, Olson AJ. *Journal of computational chemistry,* 2010; **31:**455-461.
21. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK et al. *Journal of computational chemistry,* 1998; **19:**1639-1662.

22. Yamashiro T, Yasujima T, Said HM, Yuasa H. *Journal of Biological Chemistry*, 2020; **295:**16998-7008.
23. Desmoulin SK, Wang L, Polin L, White K, Kushner J, Stout M, et al. *Molecular pharmacology*, 2012; **82:**591-600.
24. Hou Z, O'Connor C, Frühauf J, Orr S, Kim S, Gangjee A, et al. *Biochemical Journal,* 2019; **476:**1247-1266.
25. Subramanian VS, Marchant JS, Said HM. *American Journal of Physiology-Cell Physiology,* 2008; **294:**233-240.
26. Wilson MR, Hou Z, Matherly LH. *Journal of Biological Chemistry*, 2014; **289:**25287-25295.