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Original Article

INSIGHTS INTO THIORIDAZINE FOR ITS ANTI-TUBERCULAR ACTIVITY FROM MOLECULAR DOCKING STUDIES

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ABSTRACT

Objective: Thioridazine (TZ) is a drug that has been used for over 35 years as a psychoactive drug, is now potentially utilized in combination with certain anti-TB drugs to cure MDR, XDR and TDR TB. The current study explores the plausible reasons for its anti-tubercular activity through molecular docking procedure.

Methods: Molecular docking were performed by using the molecular modeling software Glide® from the suite of Schrödinger Inc., Molecular docking studies were performed to study the binding affinity of Thioridazine on the active sites of various *Mycobacterium tuberculosis* enzymes in an effort to increase the understanding of the action of Thioridazine (TZ) as an antitubercular agent. Seventeen enzymes from different mechanisms were docked and the resulting glide scores (G-Scores) were tabulated.

Results: The enzyme CmaA2 - Cyclopropane mycolic acid synthase (PDB id: 1KPI) scored lowest binding energy which means the greater stability of the Thioridazine's ability to bind to the receptor. MmaA2 (1TPY), InhA (2NSD) and PknG (2PZI) enzymes of *Mycobacterium tuberculosis* gave the best G-scores.

Conclusion: The docking study results revealed that Thioridazine may act by more than one possible mechanism to exert anti-tubercular activity against MDR (Multi Drug Resistant), XDR (Extensively Drug Resistant) and TDR (Totally Drug Resistant) -TB.

Keywords: Thioridazine (TZ), Efflux pumps, Molecular docking, Enzymes, Binding energy, MDR, XDR.

INTRODUCTION

The World Health Organization (WHO) declared TB a global public health emergency in 1993[1]. Tuberculosis (TB) still continues to be a major global health problem. Globally in 2012, an estimated 450, 000 people developed MDR-TB and there were an estimated 170, 000 deaths from MDR-T band also there was reported cases of XDR and TDR-TB. On an average, an estimated 9.6% of MDR-TB cases have converted to XDR-TB [2]. Udwadia ZF et al the clinical physicians of Hinduja Hospital, Mumbai cited the use of Thioridazine (TZ) as salvage therapy in 4 Indian patients with XDR-TB (near total drug resistance to current therapy) with advanced disease. They found the drug to be done well tolerated, even in the malnourished and ill patient population. It also led to clinical improvement in 3 of the 4 patients [3]. Thioridazine, a neuroleptic drug, which is milder and less toxic than chlorpromazine (CPZ), kills intracellular *M. tuberculosis* isolates that are resistant to two or more antibiotics. Thioridazine shows anti tuberculous effects *in-vitro* and *in-vivo* mouse models [4]. Thioridazine is effective when used in combination with antibiotics to which the initial *Mycobacterium tuberculosis* was resistant. Because Thioridazine is cheap, it should be taken into account in therapy of XDR and TDR-TB patients in economically disadvantaged countries [5, 6]. At the Hinduja Hospital (Mumbai, India), Ethical committee approval was obtained to proceed with a trial involving inclusion of Thioridazine in the treatment regimen on a 'compassionate' basis. Leonard Amaral et al [7] have now made pleas for larger collaborative studies with Thioridazine in patients with XDR-TB who have exhausted all available drug options. Van Ingen J [8] suggests that Thioridazine itself may be patented for its "new use". New drugs for treatment of mycobacterial disease, most notable multidrug- and extensively drug-resistant tuberculosis, are urgently needed; phenothiazines and their targets should be exploited for this use. The current study aims to explore the possible mechanism of action of Thioridazine through molecular docking studies. Though Thioridazine was found to act through efflux pump mechanism, still it was not well explored [9]. Present study tries to throw light on this by molecular docking studies.

MATERIALS AND METHODS

In this work Glide (grid based ligand docking with energy) program [10, 11] was used for molecular docking provided by Schrödinger Inc.,

Protein and ligand preparation

3D Crystal structures of all the enzymes reported in this work were downloaded from the Protein Data Bank (PDB) and the *in-silico* models were subjected to the Protein Preparation Wizard workflow implemented in the Schrödinger package. They were preprocessed by the addition of hydrogen, assigning the bond order, identifying overlaps, creating zero order bonds to metals and creating disulfide bonds. The co-factors, unwanted water molecules and chains were deleted. Then the energy minimization was done followed by the grid generation. Ligand was prepared by a series of steps that includes conversions, generation of variations on the structure, optimization of the structures.

Ligand docking

After the generation of the grid, prepared ligand was docked to identify the interaction between the ligand and the active site of the protein. The interactions were hydrophobic, hydrophilic and Van der Waal's interactions. The strength of ligand interaction differs from enzyme to enzyme based upon affinity. During the docking procedure, conformation of the ligand was retained and extra precision (xp) mode was selected.

Visualization of the docking results

Once the ligand was docked against all the enzymes of interest, they were visualized for interactions, G-score and some other parameters with the use of Glide XP visualiser.

RESULTS AND DISCUSSION

Comparative docking analysis of Thioridazine with various pathophysiological enzymes responsible for tuberculosis is a feasible method to study the Thioridazine potential as antimycobacterial agent. Though, Thioridazine is reported for its

efficacy towards tuberculosis in an unusual mechanism, the docking results showed it may act on other mechanisms possibly. The binding efficiency of the drug Thioridazine with 17 pathophysiological enzymes is shown in table 1 and the G-Score has ranged between -3.1 to -9.5. Hydrophobic interaction of Thioridazine when positioned at the target is shown in fig. 1. The non-bonding interactions/hydrophobic region include the key amino acid residues like tyrosine (TYR), isoleucine (ILE), leucine (LEU) and phenylalanine (PHE) in top scored three enzymes against Thioridazine.

From the virtual screening results, it is seen that Thioridazine docked well with the enzymes Cyclopropane Mycolic Acid Synthase (lipid metabolism), Methoxy Mycolic Acid Synthase (lipid metabolism), NADH-Dependent Enoyl ACP (Acyl Carrier Protein) Reductase (lipid metabolism), and Protein Kinase-G (Regulatory Protein) with the G-Score of -9.5 Kcal/mol, -9.4 Kcal/mol, -8.4 Kcal/mol and -8.2 Kcal/mol respectively. A new indication for an old antidepressant molecule Thioridazine, against *Mycobacterium tuberculosis* through an unusual mechanism has prompted the study.

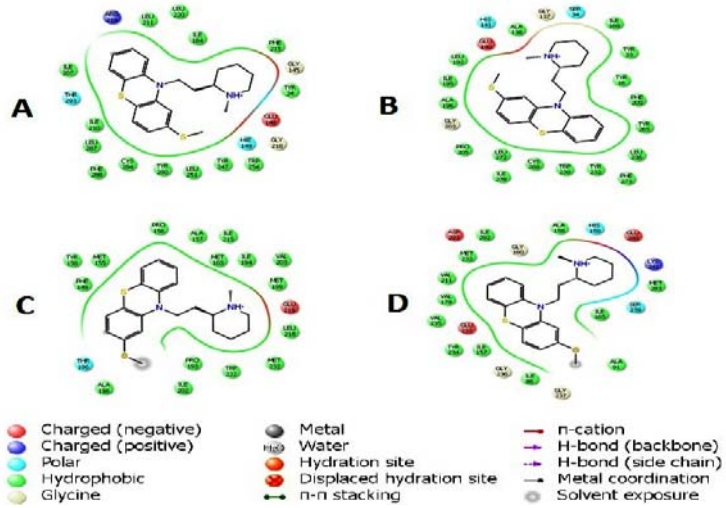

Fig. 1: Ligand Interaction diagram

A: Ligand Interaction Diagram of the CmaA2 (1KPI) enzyme with Thioridazine. B: Ligand Interaction Diagram of the MmaA2 enzyme (1TPY) with Thioridazine. C: Ligand Interaction Diagram of the InhA enzyme (2NSD) with Thioridazine. D: Ligand Interaction Diagram of the PknG enzyme (2PZI) with Thioridazine

Table 1: Molecular docking results of Thioridazine from Glide

S. No.	Name of the enzyme (PDB ID)	G-Score(Kcal/mol)	Functional category
1.	CmaA- 2 Cyclopropane Mycolic Acid Synthase[12] (1KPI)	-9.5	Lipid Metabolism
2.	MmaA2 - Methoxy Mycolic Acid Synthase 2[13](1TPY)	-9.4	Lipid Metabolism
3.	InhA - NADH-Dependent Enoyl ACP (Acyl Carrier Protein) Reductase[14] (2NSD)	-8.4	Lipid Metabolism
4.	PknG - Protein Kinase[15, 16] (2PZI)	-8.2	Regulatory Protein
5.	LdtB – L, D-Transpeptidase-2[17] (3VAE)	-6.4	Cell wall and Cell Processes
6.	GlnA1 – Glutamine Synthetase[18] (4ACF)	-6.0	Intermediary Metabolism and Respiration
7.	PcaA - Mycolic Acid (Cyclopropane) Synthase[19] (1L1E)	-5.4	Lipid Metabolism
8.	Mmr – Multi-Drug Resistant Protein[20] (2IQ4)	-4.9	Cell wall and Cell Processes
9.	AdoK – Adenosine Kinase[21] (2PKK)	-4.5	Intermediary Metabolism and Respiration
10.	Glf - UDP-Galactopyranose Mutase[22] (1V0J))	-4.1	Cell wall and Cell Processes
11.	FabH - (3-Oxo-acyl)-(Acyl Carrier Protein) ACP Synthase[23] (1HZP)	-3.7	Lipid Metabolism
12.	MbtK - Lysine-N(Epsilon)-acyl transferase[24] (1YK3)	-2.2	Lipid Metabolism
13.	FabD - Malonyl CoA- Acyl Carrier Protein transacylase [25](2QC3)	-3.8	Lipid Metabolism
14.	ThyX – Thymidylate Synthase[26] (3GWC)	-2.0	Intermediary Metabolism and Respiration
15.	TmK – Thymidylate Kinase [27](1G3U)	-3.8	Intermediary Metabolism and Respiration
16.	DprE1 - Decaprenylphosphoryl-B-D-Ribose 2′-Epimerase[28] (4FDO)	-3.8	Lipid Metabolism
17.	EmbC- Arabinosyl-Indolyl Acetyl Inositol Synthase[29] (3PTY)	-3.1	Cell wall and Cell Processes

CONCLUSION

Thioridazine as an antidepressant is reported to act by inhibiting the efflux pump mechanism. Our docking studies indicate that it might be acting by inhibition of several enzymes notably Cyclopropane Mycolic Acid Synthase (lipid metabolism), Methoxy Mycolic Acid Synthase (lipid metabolism), NADH-Dependent Enoyl ACP (Acyl Carrier Protein) Reductase (lipid metabolism), and Protein Kinase-G (Regulatory Protein) with the G-Score of -9.5 Kcal/mol, -9.4 Kcal/mol, -8.4 Kcal/mol and -8.2 Kcal/mol respectively. Therefore the Anti-TB activity of Thioridazine is not because of Efflux pump mechanism but may be by more than one mechanism possibly. Our computational studies also conclude that the novel approach on Thioridazine derivatives may lead to newer anti-tubercular agents.

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CONFLICT OF INTERESTS

We declare that there is no conflict of interest with any organization regarding the materials discussed in this work.

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