Unveiling Naturally Occurring Green Tea Polyphenol Epigallocatechin-3-Gallate (EGCG) Targeting *Mycobacterium* DPRE1 for Anti-Tb Drug Discovery [†]

Suraj N. Mali * and Anima Pandey

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra 835215, Jharkhand, India; apandey@bitmesra.ac.in

* Correspondence: mali.suraj1695@gmail.com; Tel.: +91-9657330138

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Abstract: The increasing rates of multidrug-resistant (MDR) and extremely drug-resistant (XDR) cases of tuberculosis (TB) strains are alarming, and eventually hampered the effective control of the pathogenic disease. Epigallocatechin gallate (EGCG) is a major polyphenolic constituent of green tea, which previously demonstrated in vitro potency against TB strains. However, efforts to elucidate the exact mechanism of the interaction are still ongoing. Aiming to elucidate the probable mechanism of its anti-TB action as decaprenylphosphoryl-beta-D-ribose 2'-epimerase (DprE) inhibition, we conducted a molecular modeling analysis. Our molecular docking analysis for a set of 65 bioactive compounds of tea realized that EGCG has the highest binding affinity (docking score: −142.98 kcal/mol) against DPPE (PDB ID: 4p8c) from *Mycobacterium tuberculosis*. Further, a molecular dynamics analysis of 100 ns resulted in extreme stability of the ligand–protein complex. We further assessed the in silico pharmacokinetics and toxicities of the top three green tea polyphenols, based on the docking scores. Our results provide critical insights into the mechanism of action of EGCG and other green tea polyphenols, and their use as potential therapeutic agents (DprE1) against TB.

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1. Introduction

Tuberculosis (TB) is considered to be a public health crisis, which is hampering health-care systems, especially in low-economic countries [1–5]. As per the latest WHO statistics, 1.4 million people died from TB in 2019 (10 million people fell ill) [2]. Furthermore, considering the increasing numbers of multidrug-resistant TB (MDR-TB), or multidrug- or rifampicin-resistant TB (MDR/RR-TB) cases, there is an urgent need to develop newer anti-TB agents with unique mechanisms of action. The mycobacterial cell wall is made up of mycolic acids, which are long fatty acids. The synthesis of mycolic acid is regulated via enzymes of the fatty acid synthase (FAS) complex. Enoyl reductase, pantothenate synthetase, and decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) are key attractive targets for the discovery of newer anti-TB agents. From our literature analysis, it is clear that polyphenols (Figure 1) from the leaves of green tea (GTPs) and black tea (*Camellia sinensis*) have significant pharmacological potentials against a variety of biological targets [3]. Recently, Anand et al. (2006) showed the potential of GTP, epigallocatechin-3-gallate, to inhibit *Mycobacterium tuberculosis* survival within human macrophages [5]. Their study suggested that epigallocatechin-3-gallate has the ability to down-regulate tryptophan aspartate-containing coat protein gene transcription [5]. Sun et al. (2015) proposed a probable mechanism for the effects of epigallocatechin gallate (EGCG) on the growth of *Mycobacterium smegmatis* mc(2)155 [4]. Moreover, their analysis revealed that EGCG had an impact on the integrity

of the mycobacterial cell wall. The probable targets of EGCG for the inhibition of *Mycobacterium tuberculosis* have not been studied in detail, although a few reports have mentioned some antimycobacterial activity based on enzyme inhibition studies utilizing compounds such as triclosan. Considering these facts, we particularly aimed to explore the probable anti-TB drug target for epigallocatechin gallate (EGCG), a major bioactive compound from green tea extracts [6]. Furthermore, we have also calculated docking affinity scores for other GTPs. The top three GTPs were subjected to in silico absorption, distribution, metabolism, excretion (ADME) and toxicity (T) analyses. Finally, the best docked hit, epigallocatechin gallate (EGCG), the 4P8C complex, was simulated and analyzed using 100 ns molecular dynamics analysis.

Figure 1. (a) Chemical structures of green tea polyphenols (representatives) and (b) the graphical summary of workflow followed.

Lastly, we have signified a probable lead that could be developed as a drug candidate against mycobacterial targets.

2. Materials and Methods

2.1. Molecular Docking Simulations

For the current study, we have taken a set of 65 known green tea polyphenols or compounds [7]. Overall, molecular docking comprises the following 5 main steps: (1) protein preparation, (2) ligand preparation, (3) receptor grid generation, (4) ligand docking procedure and then (5) viewing the docking results. All necessary structures have been drawn using 'ChemSketch'. All the 3D crystal structures of 3 mycobacterial proteins (the enoyl reductase receptor protein (PDB IDs: 2 × 22), decaprenylphosphoryl- β -d-ribose 2'-epimerase (DprE1), 4p8c, and the pantothenate synthetase, 3IVX) were downloaded from the protein database bank (PDB database, www.rcsb.org, accessed on 26 February 2021). The data set including the inbound (co-crystallized) ligand was docked into the binding pocket of pantothenate synthetase and DprE1 (PDB-ID: 3IVX, 4p8c) target enzymes. For the reliability of the docking protocol, we evaluated it through the RMSD value. The grid was centered around active binding site residues Gly158, Met195, Pro38, etc. (for case of 3ivx). We have performed our docking analysis using 'iGemDock' as per standard procedures by making 15 Å radius from binding pocket, followed by visualization using Discovery Studio 2020 Visualizer [8,9].

2.2. In Silico Drug Likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) Analysis

Top 3 higher-affinity GTPs were subjected to an in silico ADME analysis using SWISS tools (<http://www.swissadme.ch>, accessed on 21 April 2021). For the toxicity assessments, we used 'admetSAR' (<http://lmmd.ecust.edu.cn:8000/>, accessed on 21 April 2021).

2.3. Normal Mode Analysis

To gain more insights into the conformational flexibilities [8] of proteins with their best docked hits, we have performed normal mode analysis (NMA) with internal coordinates (IC) using a fast and easy server, iMODS (<http://imods.chaconlab.org/>, accessed on 21 April 2021).

2.4. Molecular Dynamics Studies

The docking studies do not take into account the flexible nature of the protein. For confirming the exact binding mode and stability, we have to study MD simulations with the Desmond program. The stability of epigallocatechin gallate (EGCG), 4P8C complex, was evaluated through 100 ns molecular dynamics simulations. The simulations provided the exact binding interaction of the docking complex with system embedded with water molecules, temperature and pressure. The standard NTP conditions were followed for MD simulation setup. The complex was originated in all proper binding poses with an acceptable RMSD value (<3 Å). Molecular dynamics (MD) simulation for a period of 100 ns was carried out for best docked hit with epigallocatechin gallate (EGCG), 4P8C complex, and this was achieved with the GROMACS simulation package, 2020, as per known literature protocols [9].

3. Results and Discussion

3.1. Molecular Docking Simulations

Our molecular docking analysis of 65 GTPs on 3 anti-TB targets suggested that epigallocatechin gallate (EGCG) had the highest affinity towards the DprE1 target, rather than 2X22 and 3IVX. The docking interactions depicted that this compound had interactions with GLN A:328, TYR A:60, GLY A:334, LYS A:418, VAL A:365, and LEU A:317 amino acids, with seven conventional hydrogen bonds present at the receptor site of the target 4P8C (Figure 2) [9,10]. VAL A:365 formed a pi-sigma interaction with the aromatic portion. Alkyl and pi-alkyl interactions were also observed for LEU A:363 and LEU A:317 amino acids. In Figure 2, it is also revealed that the binding mode of EGCG has favorable H-bond donors (purple colored) and acceptable (green colored) regions in the binding cavity. Tables 1 and 2 give better insights into the interaction profiles of the studied green tea/black tea molecules. From our docking analysis of 65 bioactive compounds of tea on 4p8c, we found the top three best docked hits to be theaflavin-3-gallate (docking score: -124.06 kcal/mol), epigallocatechin gallate (docking score: -142.98 kcal/mol), and epigallocatechin (docking score: -127.73 kcal/mol). The docking affinities for these three bioactive compounds were found to be greater than the standard drug ciprofloxacin* (docking score: -109.20 kcal/mol). The standard drug isoniazid interacted with LYS A:367, VAL A:365, CYS A:387, and ASN A:385 amino acid residues (docking score: -121.21 kcal/mol). The binding cavity residues for the inbound ligands were found to be VAL A: 365, LYS A: 418, ASN A: 385, and LYS A: 418 (salt bridge). Moreover, our re-docking validation protocol also resulted in an RMSD value below 2 Å, suggesting that the docking results are valid.

Figure 2. 2D and 3D interaction profiles for best docked epigallocatechin gallate with 4p8c.

Table 1. Docking interaction energies * of selected 65 bioactive molecules and 3 FDA-approved drugs for target protein 4P8C.

Molecules	-iGemDock Interaction Energy	Molecules	-iGemDock Interaction Energy
Oolonghomobisflavan A	−62.2219	Theaflavic Acid	−82.4934
Theasinensin D	−70.1619	Barrigenol R1	−86.4843
Theaflavin-3-gallate	−124.06	Barringtogenol	−52.0693
Isotheaflavin	−62.621	Camelliagenin	−95.1799
Epigallocatechin-3,5-Di-O-Gallate	−71.0176	Gallocatechin	−85.7374
Oolonghomobisflavan B	−75.4779	Catechin	−101.992
Cis-3-Hexenol	−63.5566	Epicatechin	−98.6033
Epigallocatechin-3,4-Di-O-Gallate	−92.6784	Epiafzelechin	−91.5357
Vicenin 2	−96.9806	Quercetin	−102.834
Epicatechin-3,5-Di-O-Gallate	−101.495	Cryptoxanthin	−92.1799
Rutin	−87.1416	Myricetin	−82.5936
Proanthocyanidin	−84.8129	Apigenin	−63.6163
Pheophytin	−90.2865	Nerolidol	−82.584
Benzaldehyde	−91.9877	Kaempferol	−89.1838
Epitheaflavic Acid 3′-Gallate	−65.361	Theanine	−73.9851
Epigallocatechin Gallate	−142.98	Ascorbic Acid	−80.1271
Theasinensin E	−62.6409	Quinic Acid	−75.3299
Myricitrin	−61.915	Succinic Acid	−85.5696
Theaflavin	−55.9704	Methyl Salicylate	−81.1848
Epicatechin Gallate	−72.5287	Theobromine	−72.7269
Kaempferitrin	−71.7401	Caffeine	−84.4502

Table 1. *Cont.*

Molecules	-iGemDock Interaction Energy	Molecules	-iGemDock Interaction Energy
Isoquercetin	−73.9058	Xanthine	−75.7595
Epiafzelechin 3-O-Gallate	−73.4119	Linalool Oxide	−83.9907
Pheophorbide	−71.1657	Phenylacetaldehyde	−87.8044
Epigallocatechin 3-O-P-Coumarate	−76.8643	Methylxanthine	−79.6185
Pheophorbide	−67.9266	Theophylline	−88.1319
Oxalic Acid	−82.9277	Geraniol	−95.2378
Cryptoxanthin	−81.2634	Hexanal	−95.8974
Isovitexin	−72.924	Diphenylamine	−93.4455
Vitexin	−55.6638	Trans-2-Hexenal	−94.076
Chlorogenic Acid	−49.7604	Linalool	−86.4307
Coumaroyl Quinic Acid	−94.7189	Phenylethanol	−101.468
Epigallocatechin	−127.73	Ciprofloxacin *	−109.20

* Docking scores have only been provided for the higher-affinity scored target protein.

Table 2. Energy contribution of the key residues computed by docking methodology.

Sr. No.	Molecules	Residues with Contribution Energy
1.	Isoniazide	LYS A:367; VAL A:365; CYS A:387; ASN A:385
2.	Pyrazinamide	VAL A: 365; LYS A: 418; ASN A: 385
3.	Ciprofloxacin	GLN A:117; VAL A:365; CYS A:387; LYS A:367; HIS A:132
4	Theaflavin-3-gallate (Best docked)	TRP A:16; LEU A:363; HIS A:315; THR A:118; LEU A:317
5	Epigallocatechin	GLN A:334; TYR A:60; CYS A:387; LYS A:418
6	Epigallocatechin Gallate (EGCG)	GLN A:328; TYR A:60; GLY A:334; LYS A:418; VAL A:365; LEU A:317
7	Inbound ligand (y22)	VAL A: 365; LYS A: 418; ASN A: 385; LYS A: 418 (salt bridge)

3.2. Molecular Dynamics Simulation and Normal Mode Analysis

The best docked hit, epigallocatechin gallate (EGCG), with the target 4P8C, was simulated for 100 ns molecular dynamics and normal mode analysis. The MD simulations showed that the obtained root mean square fluctuation (RMSF) values were within tolerable ranges (0.4 nm). The obtained root mean square deviation (RMSD) value was below 0.25 nm, suggesting that the complex was stable (Figure 3). From our NMA results, we observed that epigallocatechin gallate, with the protein 4P8C complex, was retained with good deformability and eigenvalue profiles (Figure 3). The radius of the gyration value was also below 2.15 nm. The accessible surface areas of the solvent were found to be around 180 nm².

Figure 3. (a) The binding pocket; (b) the root mean square deviations (RMSD) of backbone atoms relative to the starting complexes during 100 ns MD; (c) protein RMSF plot (on this plot, peaks indicate areas of the protein that fluctuate the most during the simulation, and protein residues that interact with the ligand are marked with green-colored vertical bars); (d) deformability and (e) B-factor profiles; (f) eigenvalue and (g) covariance matrix of the complex for epigallocatechin gallate with 4p8c, respectively.

3.3. In Silico ADMET Studies

Cytochrome P450 (CYPs) enzymes are key enzymes responsible for various factors of metabolism. Our in silico calculated ADMET (absorption, distribution, metabolism, excretion, and toxicity) values, in the body properties evaluated, for the top three best docked hits are represented in Table 3. Three GTPs, theaflavin-3-gallate, epigallocatechin, and epigallocatechin gallate (EGCG), exhibited positive human intestinal absorption profiles, negative blood–brain barrier passage, and non-carcinogenic, non-AMES toxicity, and class IV acute oral toxicity profiles.

Table 3. In silico ADMET profiling for top 3 best docked hits against target 4P8C.

Properties	Theaflavin-3-Gallate	Epigallocatechin	Epigallocatechin Gallate (EGCG) *
CYP450 2C9 Substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 Substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 Substrate	Non-substrate	Non-substrate	Non-substrate
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	Weak inhibitor	Weak inhibitor
AMES Toxicity	Non-AMES toxicity	Non-AMES toxicity	Non-AMES toxicity
Carcinogens	None	None	None
Acute Oral Toxicity	IV	IV	IV
P-glycoprotein Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Rat Acute Toxicity (LD ₅₀ , mol/kg)	2.6693	1.8700	2.6643
Human Intestinal Absorption	+	+	+
AlogP	3.19	1.25	2.23
H-Bond Acceptor	16	7	11
H-Bond Donor	11	6	8
Tetrahymena pyriformis (pIGC50 (ug/L))	0.595	0.792	0.913
Blood–Brain Barrier	-	-	-

* Best docked.

4. Conclusions

In our current study, we noticed that epigallocatechin gallate (EGCG) has strong interactions with the 4p8c enzyme (docking score: -142.98 kcal/mol), and the following amino acid residues: GLN A:328; TYR A:60; GLY A:334; LYS A:418; VAL A:365; LEU A:317. The binding energy obtained for EGCG was higher when compared with the standard drug ciprofloxacin (docking score: -109.20 kcal/mol). Moreover, in silico ADMET analysis revealed that this compound has low human ether-a-go-go-related gene inhibition, no AMES toxicity, and no carcinogens. Considering a previous literature report on EGCG as an antimycobacterial, we investigated the same against the DprE1 enzyme via molecular docking analysis. Considering the benefits of GTPs, this study may provide further directions to develop more potent anti-TB compounds. Moreover, we also believe that synthetic analogues of EGCG could be tested for in vitro anti-TB potentials. The tea extract containing EGCG could also be tested in vitro for anti-TB enzymatic assessments.

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