ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 6, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

NEUROPROTECTIVE ROLE OF BIMOCLOMOL IN ECTOPIC CELL CYCLE IN PARKINSON'S DISEASE: NEW INSIGHTS

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Received: 17 February 2017, Revised and Accepted: 11 March 2017

ABSTRACT

Objective: Parkinson's disease (PD) is a debilitating age-related neurodegenerative disease characterized by the canonical formation of intracellular Lewy bodies comprising α-synuclein protein. Despite the knowledge of factors causing PD, it remains irreversible and incurable. Recent studies have highlighted the physiological and pathological involvement of cell cycle proteins in PD. The intriguing relationship between PARK2 and cyclin E which leads to upregulation of cyclin E in the absence of functional PARK2 contributes heavily in the onset and progression of PD. The objective of this study is to explore neuroprotective action of bimoclomol in attenuating the level of cyclin E and inhibiting post-mitotic cell division led neurodegeneration in PD.

Methods: We employed various *in silico* methods such as drug-likeness parameters, namely, Lipinski filter analysis, Ghose parameters, Veber rules, absorption, distribution, metabolism, and excretion - toxicity analysis, pharmacophore based target prediction, active site prediction, and molecular docking studies.

Results: The binding of bimoclomol inhibited cyclin E, thereby, attenuating post-mitotic cell division led neurodegeneration in PD.

Conclusion: This study outlines the novel potential of bimoclomol in attenuating cyclin E led neuronal death in PD which may be mediated by heat shock proteins (HSP70).

Keywords: Parkinson's disease, Bimoclomol, Cell cycle, Heat shock proteins 70, Therapeutics.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i6.17971

INTRODUCTION

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder affecting 2% of population aged above 65 years in industrialized nations. It is characterized by pathogenic protein burden and intracellular inclusion body formation, namely, Lewy bodies and Lewy neurites constituted by α-synuclein protein. The clinical cardinal features of PD include resting tremor, bradykinesia, postural instability and rigor, often accompanied by impaired cognition [1]. These symptoms are an outcome of dopaminergic neuronal loss in the substantia nigra pars compacta region of the brain.

Genetic and animal studies have outlined various causative phenomenon in PD including mutations in genes predominantly; Parkin, α-synuclein, PINK1 and DJ-1, oxidative stress, aging, impaired ubiquitin proteasome system, and dysfunctional mitochondrial system. Despite the availability of this knowledge, the etiology of PD remains incurable and irreversible. Moreover, recent studies have highlighted the involvement of aberrant cell cycle in PD [2]. Interestingly; cell cycle proteins share a very intimate relationship with proteins of PD, physiologically as well as pathologically. The PD-associated gene; PINK1 was shown to promote cell cycle, and PINK1 deletion reversed cell proliferation [3]. Further, the ATM gene responsible for DNA damage response and apoptosis has been found to be activated along with retinoblastoma protein leading to neuronal death in MPP^+ induced PD model [4]. However, the most crucial and strong correlation between cell cycle and PD is provided by the association of cyclin E and PARK2. Cyclin E is G_1/S phase marker of the cell cycle and also a substrate of ubiquitin E3 ligase PARK2. Mutations associated with loss of functional PARK2 are linked with cyclin E enrichment led cell cycle and apoptosis through p53 and Bax in PD. Further, mutated PARK2/cyclin E events evoked upregulation of Wnt/β catenin and EGFR/AKT signal transduction pathways [5]. This intriguing PARK2/cyclin E relation led to speculations that compounds which can bind to and attenuate the level of cyclin E can ameliorate post-mitotic cell division led neurodegeneration in PD.

Numerous studies have highlighted the neuroprotective action of heat shock proteins (HSP) particularly, HSP70. Further, HSP70 is closely associated with cell cycle regulation and was also found to interact with cyclin E in inclusion body myositis and polymyositis [6]. Therefore, we carried out comprehensive data mining for HSP70 inducers in neurodegenerative diseases and selected bimoclomol among 20 compounds based on drug-likeness, pharmacokinetics, and blood–brain barrier (BBB) permeability (unpublished results). Bimoclomol is a hydroxylamine derivative with molecular formula $C_{14}H_{20}ClN_3O_2$ and molecular weight of 297.783 g/mol. It has been shown to elicit protective effects through induction of HSP27, HSP70, and HSP90. Moreover, bimoclomol is currently under Phase II trials in amyotrophic lateral sclerosis disease [7]. Therefore, we investigated the neuroprotective action of bimoclomol through attenuation of cyclin E in PD. Various virtual screening methods such as Lipinski filter, Ghose and Veber parameters, pharmacophore modeling based target prediction, and ADME analysis were employed to check the efficacy of bimoclomol as a neuroprotective agent. Further, we studied the cyclin E inhibiting potential of bimoclomol through molecular docking studies.

METHODS

Retrieval of ligand-protein structure

The SDF file of bimoclomol was retrieved from the PubChem database (http://www.pubchem.ncbi.nlm.nih.gov/). The pubChem database stores physiochemical and biological information of compounds from

three different databases. The protein data bank (PDB) file of cyclin E was retrieved from PDB (http://www.rcsb.org/pdb/home/home.do).

Drug-likeliness analysis

The drug-likeness of bimoclomol was tested through Lipinski filter analysis which is used to test compounds for drug ability. The Lipinski's rule of five is: (a) Molecular mass <500 Dalton, (b) lipophilicity (logP) <5, (c) hydrogen bond donors <5, (d) hydrogen bond acceptors <10, and (e) molar refractivity between 40 and 130 [8]. In addition, two other parameters; Ghose filter and Veber rules were employed for drug-likeness screening (www.swissadme.ch/index.php). The qualifying parameters of Ghose filter are (a) molecular weight 160-480, (b) number of atoms 20-70, (c) molar refractivity 40-130, (d) molar refractivity - 0.4-5.6, and (e) polar surface area <140 [9]. The Veber rules are (a) rotatable bond count ≤10 and (b) polar surface area ≤140 [10].

Absorption, distribution, metabolism, and excretion - toxicity (ADMET) analysis

The toxicity profiling of bimoclomol was carried out through the online tool Swiss ADME (www.swissadme.ch/index.php). The Swiss ADME tool assessed the ligand on various parameters such as logP, hydrophilic nature (logS), and BBB permeability.

Pharmacophore based target prediction

A pharmacophore is a spatial arrangement of steric and electronic properties of a compound responsible for its biological response against a particular target. Pharmacophore-based target prediction of bimoclomol was done with web server PharmMapper (http://59.78.96.61/pharmmapper/index.php) [11].

Active site prediction

The active sites of cyclin E were predicted using the pockdrug tool (http://pockdrug.rpbs.univ-paris-diderot.fr/cgi-bin/index.py?page=home) [12]. The PDB structure of cyclin E was uploaded, and active sites were predicted using fpocket estimation and setting ligand proximity threshold at 5.5.

Preparation of protein and ligand for docking

Cyclin E and bimoclomol were prepared for docking using the online docking server (http://www.dockingserver.com/web) [13]. The protein was cleaned, and chain A of cyclin E was selected for docking. The protein and ligand charge was calculated using Gasteiger method, and default solvation parameters were set. The ligand geometry was optimized using the MMFF94 method. Further, all non-polar H_2 atoms were merged, rotatable bonds defined and pH set to 7.0.

Molecular docking

The optimized proteins and ligands were used for molecular docking studies using the online docking server (http://www.dockingserver.com/web). The Autodock tool was used for adding Kollman united atom type charges, essential H_2 atoms, and solvation parameters. Affinity grid maps were generated with 0.375 Å spacing [14]. Further, the van der Waals and electrostatic interactions were calculated using Autodock parameter set and distance-dependent dielectric functions, respectively. Furthermore, the Lamarckian genetic algorithm and Soils and Wets local search method was used for docking simulations [15]. During docking, all rotatable torsions were dropped. Every docking study was arrived after 10 different runs with a cut off energy estimation of 250000. Finally, translational step with 0.2 Å, torsion and quaternion steps of five were used with a population size of 150.

RESULTS

Protein-ligand structure

The 3D structures of bimoclomol and cyclin E were retrieved from docking server and PDB, respectively (Fig. 1).

Screening for drug-likeness and ADMET analysis of compounds

Bimoclomol passed all the parameters related to drug-likeness screening, namely, Lipinski, Ghose and Veber. Most importantly, it can cross the BBB and has high pharmacokinetics values (Table 1).

Pharmacophore based target prediction

The pharmacophore based target prediction of bimoclomol revealed mitogen-activated protein kinase 14 as one of the top 10 targets with a fit score of 3.521 and Z-score value of −0.408482 which supported our premise of its strong potential in inhibiting cell cycle (Fig. 2).

Active site prediction

Out of top 10 pockets, cyclin E had best pocket at P20 with a drug ability score of 0.94 and 0.01 standard deviation (Fig. 3). The volume of given pocket was 551.26 cubic angstroms and 14 residues were involved in interaction at this site.

Molecular docking of bimoclomol with cyclin E

Bimoclomol bound to cyclin E at P20 pocket and same residues as predicted were involved in the interaction (Fig. 4). The estimated free energy of binding for cyclin E and bimoclomol was −5.07 kcal/mol, and total intermolecular energy was −6.48 kcal/mol (Table 2). There were two H_2 bond formations involving GLU188 and LYS186 with bond energies of −0.2603 kcal/mol and −0.2271 kcal/mol, respectively. Further, a hydrophobic bond was formed with HIS147 (−0.6293 kcal/mol).

DISCUSSION

PD is the second most common age-related neurodegenerative disease affecting those aged above 60 years. Despite the knowledge of several factors which contribute in the occurrence and progression of PD, the exact cause and cure remain elusive. Ectopic activation of the cell cycle in terminally differentiated neurons is a recently known phenomenon which has been shown to drive neurodegeneration through actual DNA

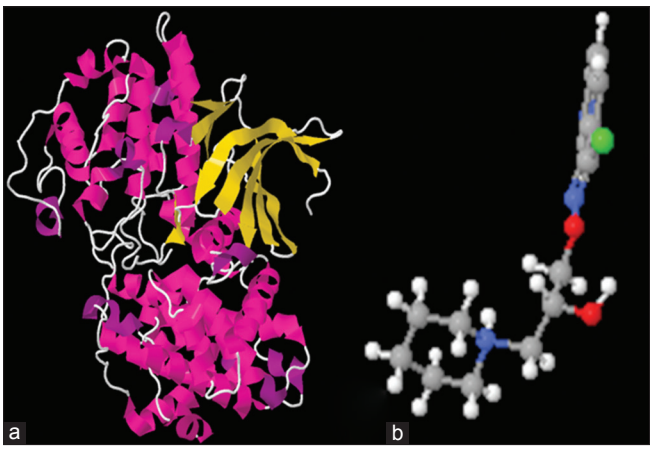

Fig. 1: Three-dimensional structure of cyclin E (a) and bimoclomol (b)

Ligand: 9576891

	Rank	PDB ID	Target Name	Number of Feature	Fit Score	Normalized Fit Score	z'-score
+	1	1ONG	Beta-lactamase SHV-1	4	3.548	0.8871	0.0454138
+	2	1DY4	Exoglucanase 1	4	3.525	0.8812	0.288696
+	3	1GCZ	Macrophage migration inhibitory factor	4	3.409	0.8523	-0.339281
+	4	2B55	Cell division protein kinase 2	5	3.802	0.7605	1.62195
+	5	2BTO	NONE	5	3.789	0.7578	1.49587
+	6	1Q6I	FKBP-type peptidyl-prolyl cis-trans isomerase fkpA	5	3.7	0.74	1.15025
+	7	3CPA	NONE	5	3.544	0.7087	-0.0490846
+	8	2ZAZ	Mitogen-activated protein kinase 14	5	3.521	0.7042	-0.408482
+	9	2UWL	Coagulation factor X	5	3.394	0.6788	-0.426764
+	10	3MDE	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	6	3.99	0.665	0.078465

Fig. 2: Pharmacophore based target prediction of bimoclomol

Pockets	Vol. Hull*	Hydroph. Kyte*	Polar Res.*	Aromatic Res.*	Otyr atom	Nb. Res.*	Drugg Prob*	Standard Deviation
P 0	2058.21	-0.36	0.59	0.17	0.0	29.0	0.72	0.06
P 1	2863.51	-1.49	0.71	0.26	0.03	31.0	0.26	0.01
P 15	634.09	0.15	0.53	0.2	0.0	15.0	0.88	0.04
P 17	642.52	0.21	0.4	0.07	0.0	15.0	0.82	0.02
P 2	852.24	0.12	0.41	0.06	0.0	17.0	0.77	0.03
P 20	551.26	0.73	0.29	0.07	0.0	14.0	0.94	0.01
P 3	1146.05	0.04	0.45	0.1	0.0	20.0	0.78	0.01
P 4	1067.3	-0.88	0.67	0.11	0.0	18.0	0.29	0.01
P 5	674.13	-0.63	0.63	0.13	0.0	16.0	0.42	0.02
P 9	1030.55	-0.64	0.57	0.14	0.0	14.0	0.46	0.03

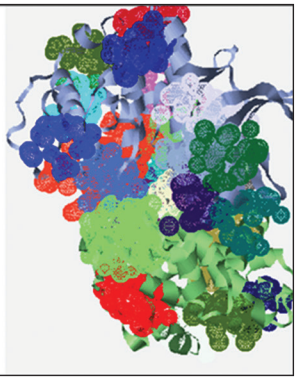

Fig. 3: Active sites of cyclin E

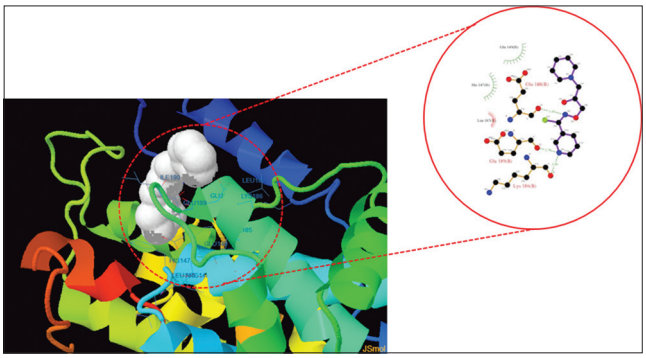

Fig. 4: Docking of bimoclomol with cyclin E and residues involved (inset)

Table 1: ADMET analysis of Bimoclomol

GI permeability	High
LogS (ESOL)	−2.9
XLogP3	2.21
Bioavailability score	0.55

ADMET: Absorption, distribution, metabolism, and excretion - toxicity, GI: Gastrointestinal, ESOL: Estimated aqueous solubility

Table 2: Energies of binding for cyclin E and bimoclomol

Estimated free energy of binding	−5.07 Kcal/mol
Estimated inhibition constant	191.27 uM
vdW+Hbond+desolv energy	−4.55 Kcal/mol
Electrostatic energy	−1.93 Kcal/mol
Total intermolecular energy	−6.48 Kcal/mol
Interacting surface	541.668

synthesis followed by apoptosis [2]. Moreover, PD-associated proteins have shared a very intimate relation with cell cycle markers. The G1/S phase marker cyclin E is a substrate for PARK2 and participates in ubiquitination process. However, mutations in PARK2 led to the loss of function thereby, resulting in cyclin E accumulation which in turn, activated E2F1 and triggered neuronal death in PD [5]. Thus, it seems imperative to design therapeutic strategies aimed at attenuating the level of cyclin E to inhibit the cascade of neuronal death in PD.

Various biomolecules such as curcumin elicited HSP70 activity and provided protection against neuronal dysfunction, various cancers, and in vascular diseases [16-18]. Bimoclomol is a hydroxylamine derivative which is nontoxic and elicited its protective effect through HSP induction; including HSP70 [19]. HSPs are molecular chaperones which are upregulated in the cell to protect it against heat, ROS and hypoxia. HSP70 has been shown to promote neuronal survival by mediating the activation of pro-survival signaling cascades and through autophagy induction [2]. Interestingly, HSP70 has been shown to interact with cyclin E in Aβ induced cell cycle re-entry in inclusion body myositis and polymyositis [6]. Taken together, all these data provide convincing evidence of using HSP70 inducing compound such as bimoclomol in attenuating the level of cyclin E and in turn, inhibit the cascade of neuronal death in PD.

In this study, we tested the drug-able efficacy of bimoclomol for targeting cyclin E in PD. Emphasis was laid on pharmacokinetic analysis as aqueous solubility, and dissolution in gastrointestinal fluids are defining parameters of *in vivo* bioavailability of an orally administered drug [20]. Similarly, the lipophilicity of a drug directs physiological properties such as rate of metabolism, transport across cell membrane and interaction with binding sites of the receptor. Further, CNS drugs should have logP <4 [21,22]. The logP value for bimoclomol was found to be 2.21.

However, the most important property required of a compound intended to be a neuroprotective agent is BBB permeability. Bimoclomol qualified all the above-mentioned parameters and scored well on pharmacokinetics, bioavailability score and could cross the BBB. Finally, molecular docking studies indicated that bimoclomol can bind to and attenuate the level of cyclin E and possibly, halt or inhibit cell cycle re-entry mediated neuronal death in PD. These findings can be validated through *in vitro* and *in vivo* cell cycle studies in PD.

CONCLUSION

The results of our study provide the novel potential of bimoclomol in attenuating the level of cyclin E which has wider implications in inhibiting cell cycle re-entry mediated neurodegeneration in PD.

ACKNOWLEDGMENT

The authors would like to thank senior management of Delhi Technological University for their encouragement and support. The authors would also like to thank University Grants Commission for providing Senior Research Fellowship to R.S.

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