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Research Article

Identification of Targeted Therapeutic Compounds for the Treatment of Traumatic Brain Injuries: An in-Silico Study

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Abstract

Introduction: Traumatic Brain Injury (TBI) is a significant public health problem. Detecting effective therapeutic compounds for the management of TBI patients is essential. Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor that regulates oxidative stress and inflammation after TBI. This study aimed to assess Targeted Therapeutic Compounds by inhibiting the degradation of Nuclear Factor E2-Related Factor 2 for TBI management.

Methods: The compounds that inhibit Nrf2 degradation by suppressing Nrf2 inhibitors, such as Kelch-like ECH-associated protein 1 (Keap1), E3 ligase adapter β-TrCP, and glycogen synthase kinase 3 (GSK-3), were identified by a literature review. Possible active sites for identified target proteins were determined. Molegro Virtual Docker (MVD) software predicted the protein-ligand interaction study. The interaction compounds that inhibit Nrf2 degradation (Keap1, GSK-3, and β-TrCP proteins) with over 274000 drug-like compounds from the ZINC database were analyzed by molecular docking. Selected compounds from the molecular docking analysis were subjected to ADME prediction for pass drug parameters. The molecules were imported to Swiss Similarity software, and screening was performed using the Food and Drug Administration (FDA) approved drugs library. To assess the structural stability of the hit compounds, molecular dynamics (MD) simulation using the GROMACS package version 2020.1 was performed.

Results: Four molecules, including ZINC435885061, ZINC469515563, ZINC119772060, and ZINC550706912, can suppress all three Nrf2 inhibitors and are capable of penetrating the blood-brain barrier. The ADME analysis of the compounds showed that these four molecules could be used therapeutically. Nebivolol is an FDA-approved drug identified for neuroprotective effects and can be used as a potential candidate for treatment.

Conclusion: Targeted therapeutic compounds that inhibit Nrf2 degradation can be used for TBI treatment. Nebivolol, an FDA-approved drug, has neuroprotective effects and can be used for TBI treatment, but further studies, such as animal studies and clinical trials, are necessary.

Keywords: Traumatic brain injury, nuclear factor E2-related factor 2 (Nrf2), virtual screening, molecular docking, Nebivolol.

Introduction

TBI and Mild Traumatic Brain Injury (mTBI) are significant global problems. Also, TBIs are related to chronic traumatic encephalopathy, memory deficits, Post-Traumatic Stress Disorder (PTSD), and chronic neuro-inflammation [1].

mTBI is prevalent in vehicular accidents, sports-related injuries, and military trauma injuries [2].

The inflammation after TBI is known as an interaction between peripheral and central cellular and soluble components, affected by patient sex, age, degree of injury (mild, severe), mechanism of injury (blast, diffuse, focal), secondary insults (hypoxemia, hypotension), and genetic diversity. Post-traumatic inflammation can be advantageous since it stimulates regeneration and debris clearance. Also, it can be potentially disadvantageous by facilitating neuronal death [3-11]. Therefore, optimizing the inflammation and, principally, immune response to TBI is crucial for immune targeting interventions in TBI. This optimization stimulates an anti-inflammatory effect and inhibits the progression of chronic neuroinflammation.

Generally, the development from acute inflammation to chronic inflammation and the progress of traumatic encephalopathies were usually interested in drug design, especially targeted drugs that can balance and promote inflammation's advantages for the regenerative process. The effectiveness of pharmaceutical agents used in the treatment of TBI is low. Therefore, screening novel therapeutic compounds is necessary [3]. A change in gene expression in the affected area of TBI can occur. Lipponen et al. showed that the Nfe2l2 gene or Nuclear factor E2-related factor 2 (Nrf2) could be a therapeutic target in this disease [3]. Nrf2 is a transcription factor considered one of the primary regulators of oxidative stress. Under normal conditions, the level of Nrf2 in the cell cytoplasm is mainly controlled by the Kelch-like ECH-associated protein 1 (Keap1) [4]. In contrast, under oxidative stress, Nrf2 is activated and induces the expression of target genes. Nrf2 regulates the expression of about 1055 genes with an antioxidant response element (ARE) sequence in their promoter region [5]. These genes are involved in antioxidant, cellular proliferation, immune response, cell survival, metabolism, and signaling. Nrf2 can be targeted pharmacologically in diseases affected by oxidative stress and inflammation, such as TBI. Activation of Nrf2 leads to an antioxidant and anti-inflammatory response in the cell. Nrf2 is regulated at the posttranslational level by proteasomal degradation. Keap1 is the main factor in this process; however, βTrCP and Hrd1 can induce proteasomal degradation of Nrf2 [6]. Keap1 binds to the Nrf2 and prepares the Nrf2 for degradation by the E3 ligase complex formed by Cullin3 and RBX1 proteins (CUL3/RBX1). Another mechanism for proteasomal degradation of Nrf2 is performed by the glycogen synthase kinase 3 (GSK-3) and the E3 ligase adapter β-TrCP. GSK-3 phosphorylates the Nrf2, and the β-TrCP is called to the site. Then, this complex is ready to be degraded by the CUL3/RBX1 complex [7, 8].

Therapies are targeted for TBI based on responsive inflammation occurring at different times. The release of reactive oxygen species and damage-associated molecular patterns triggers inflammation after TBI.

Consequently, the Nrf2 can be targeted pharmacologically to optimize inflammatory and oxidative stress responses after TBI. The therapeutic compounds can be identified to inhibit Nrf2 degradation by suppressing Keap1, β-TCP, and GSK-3, and finally, optimize the inflammation responses using virtual screening. This study aimed to assess Targeted Therapeutic Compounds by Inhibition of Degradation of Nuclear Factor E2-Related Factor 2 for managing TBIs.

Methods

Virtual screening and molecular docking

A literature review identified the Keap1, GSK-3, and β-TrCP proteins as inhibitor compounds for Nrf2 degradation. The 3D structures of Keap1, GSK-3, and β-TrCP proteins were obtained from the RCSB Protein Data Bank (PDB, https://www.rcsb.org/) with PDB IDs 1U6D, 4AFJ, and 6M90, respectively. Molegro Virtual Docker (MVD) software was used to prepare the input files and analyze the PDB files.

The 3D structures of drug-like molecules were downloaded from the ZINC database. The selection of compounds from the ZINC database was based on the size of Keap1, previously known as inhibitor compounds for Nrf2 degradation in the literature review; molecular weight in the range 375 to 500, Log P in the range 4 to 5, neutral charge, and mild pH were used as selection parameters for compounds from the ZINC database. Based on this, 274000 compounds were randomly acquired. Possible active sites for the target proteins (Keap1, GSK-3, and β-TrCP) were determined, and MVD was used to predict the protein-ligand interaction study. At first, the 274000 compounds were docked with the Keap1 protein; then, the docking results

were docked with the β-TrCP protein and the GSK-3 proteins. MolDock score function, based on the piecewise linear potential (PLP), was used to evaluate the docking results [9].

ADME and similarity prediction

Selected compounds from the molecular docking analysis were subjected to ADME prediction for pass drug parameters. The selected compounds ' pharmacokinetic parameters, such as absorption, distribution, metabolism, and excretion (ADME), were analyzed using the SwissADME web tool [10]. Also, the SwissSimilarity free web tool was applied for similarity prediction [11]. Selected molecules were imported to the SwissSimilarity software, and screening was performed using the Food and Drug Administration (FDA) approved drugs library. Five methods were applied to screen the library of FDA-approved drugs, including fingerprints, Electroshape, Spectrophores, Shape-IT, and Align-IT. Then, the commonly screened molecules from at least three methods were selected.

Molecular dynamics simulation

The assessment of the structural stability related to the hit compounds, molecular dynamics (MD) simulation using the GROMACS package version 2020.1, was performed. The docking study selected the desired protein/ligand complexes as an input file for MD simulations. Topology files were generated by the PRODRG server [12]. The GROMOS96 54A7 force field was used. A Cubic box filled with a TIP3P water model surrounded the complex. Neutralization of the system was performed. After energy minimization, temperature, and pressure equilibration, the particle mesh Ewald (PME) method was used to calculate long-range electrostatic interactions. 0.9 Å cut-off was fixed for non-bonded Van der Waals interactions and a short-range electrostatic cut-off. The bond lengths were constrained using the LINCS algorithm. The modified Berendsen thermostat (V-rescale) and Parrinello–Rahman barostat were used to keep the temperature and pressure constant at 300 K and 1 bar. Finally, root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration were analyzed.

Results

Docking of ligands

To screen compounds that inhibit Nrf2 degradation, molecular docking was performed on over 274000 drug-like compounds from the ZINC database on the Keap1 protein. The results were sorted based on the MolDock score, and 9314 compounds were selected with docking scores lower than -130 KJ/mol. The top 50 of these compounds are listed in Table 1 of the supplementary data. In the second step, the preferred compounds (9314 compounds) were applied to molecular docking on the β-TrCP protein; the results were sorted from lowest to highest MolDock score. One thousand seven hundred eighty-six compounds with docking scores lower than -130 KJ/mol were screened; the top 50 with the lowest docking score are shown in Table 2 of the supplementary data. Finally, molecular docking was performed for 1786 compounds on the GSK-3 protein, and the results were sorted based on the acquired MolDock score. The top 50 compounds are demonstrated in Table 3 of the supplementary data.

ADME and similarity prediction

The SwissADME web tool was applied to predict pharmacokinetic parameters, such as absorption, distribution, metabolism, and excretion (ADME). Compounds selected from the docking study for ADME analysis were applied (Tables 4,5, and 6 of supplementary data). The blood-brain-barrier (BBB) penetration was necessary for this drug-like compound screening. Therefore, four compounds, including ZINC435885061, ZINC469515563, ZINC119772060, and ZINC550706912, could penetrate the blood-brain-barrier (BBB) and were selected. Other features, such as molecular weight, aqueous solubility, human intestinal absorption, etc., were calculated for all selected compounds (Tables 1-6 of supplementary data).

To find an FDA-approved drug, the library-related drugs were screened against ZINC435885061, ZINC469515563, ZINC119772060, and ZINC550706912, which were obtained from previous steps. The common molecules were selected in at least three screening and data methods (Table 1).

Screening against ZINC469515563 returned Betaxolol and Nebivolol as similar molecules. Similarity screening against ZINC119772060 returned Levocabastine. Buprenorphine was obtained from screening against ZINC119772060, and screening against ZINC435885061 returned nothing. Molecular docking results of these compounds on the Keap1, β-TrCP, and GSK-3 are demonstrated in Table 2.

Molecular dynamics simulations

The molecular dynamics simulations assessed the structural stability of Keap1-ZINC469515563, Keap1-ZINC550706912, and Keap1-Nebivolol complexes in the physiological and environmental conditions. RMSD, RMSF, and Radius of gyration were analyzed in this study. The Root Mean Square Deviation (RMSD) for Keap1-ZINC469515563, Keap1-ZINC550706912, and Keap1-Nebivolol complexes were within 0.1 nm to 0.25 nm, 0.1 nm to 0.25 nm, and 0.1 nm to 0.23 nm, respectively. Also, the average RMSD for Keap1-ZINC469515563 was 0.18 nm, 0.20 nm for the ZINC550706912 ligand, and Nebivolol was 0.19 nm (Figure 1). The RMSF was applied to determine the fluctuations and flexibility of each residue in Keap1 during the last ten ns simulation period. The average RMSF for Keap1-ZINC469515563, Keap1-ZINC550706912, and Keap1-Nebivolol complexes were 0.11 nm, 0.07 nm, and 0.09 nm, respectively (Figure 2). The radius of gyration for Keap1-ZINC469515563, Keap1-ZINC550706912, and Keap1-Nebivolol complexes was within 1.69 nm to 1.74 nm, 1.7 nm to 1.75 nm, and 1.69 to 1.74 nm, respectively (Figure 1 of supplementary data). These results demonstrated that all of the selected compounds were stable.

Table 1:Similarity screening results of screened compounds

		Similarity screening methods				
Molecule	**FDA approved drug**	**FP2 fingerprints**	**Electroshape**	**Spectrophores**	**Shape-IT**	**Align-IT**
ZINC119772060	Levocabastine	-	0.706	0.524	0.777	-
ZINC469515563	Betaxolol	-	0.734	0.555	0.771	-
	Nebivolol	-	0.706	0.636	0.775	-
ZINC550706912	Buprenorphine	-	0.801	0.83	0.753	-

Table 2: Molecular docking results of screened FDA approved drugs.

	KEAP1		**β-TrCP**		**GSK3β**	
	mol dock score (KJ/mol)	**Rerank score**	**mol dock score (KJ/mol)**	**Rerank score**	**mol dock score (KJ/mol)**	**Rerank score**
Nebivolol	-88.3114	-61.2271	>0.000	>0.000	>0.000	>0.000
Betaxolol	-85.837	-50.401	-86.6224	-46.6435	-87.2839	-63.0346
Levocabastine	-42.4964	22.753	-53.7759	-7.19937	-68.3468	-36.9315
Buprenorphine	>0.000	>0.000	>0.000	>0.000	>0.000	>0.000

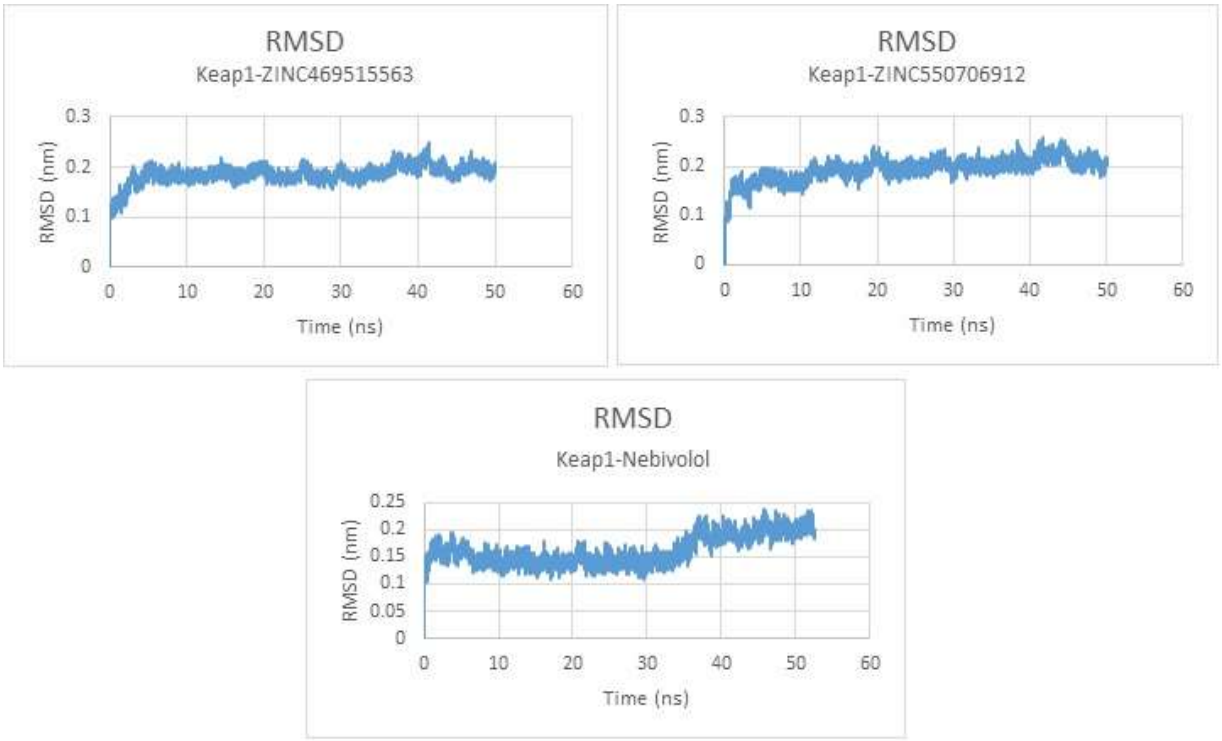

Figure 1: RMSD plots of Keap1-ZINC469515563, Keap1-ZINC550706912 and Keap1-Nebivolol complexes

Discussion

Nrf2 can be used as a therapeutic target for TBI [3]. This molecule for drug discovery was exposed in this in silico study. The virtual screening was used to identify compounds that inhibit Nrf2 degradation by suppressing Nrf2 inhibitors such as Keap1, β-TrCP, and GSK-3. The results showed that ZINC435885061, ZINC469515563, ZINC119772060, and ZINC550706912 could suppress all three Nrf2 inhibitors, and also these four molecules can penetrate the blood-brain barrier. ADME analysis of these four molecules identified that these compounds could be used as therapeutic compounds. However, these compounds have not been included in the clinical trial phase. In the next step, similarity screening was performed to find similar FDA-approved drugs with these four molecules.

Similarity screening against these four molecules returned Betaxolol, Nebivolol, Levocabastine, and Buprenorphine. As a beta (1)-adrenergic receptor antagonist, Betaxolol is used to treat hypertension and elevated intraocular pressure. Betaxolol selectively blocks beta (1)-adrenergic receptor in the heart and vascular smooth muscle, reducing heart rate, cardiac output, and systolic and diastolic blood pressure [13]. Ramos et al. showed that Betaxolol could improve prefrontal cortex (PFC) cognitive functions, such as working memory performance, in rats and monkeys [14]. Some meta-analysis studies showed that beta-blocker treatment reduces mortality after TBI [15-17]. Levocabastine, a selective histamine H1 receptor antagonist, can manage seasonal allergic symptoms [18]. Buprenorphine is a partial mu-opioid receptor agonist and can be used for severe pain and opioid dependence management [19]. A study found that Buprenorphine could protect the neurons in the thalamic reticular nucleus's medial and lateral regions in patients resuscitated from cardiac arrest [20]. Ryu et al. showed that treating TBI with Buprenorphine in rats affects acute glial pathology at 1-day post-injury but does not affect axonal injury [21]. Nebivolol is a third-generation beta-blocker agent with high selectivity for beta (1)-adrenergic receptors and is used for cardiovascular disease management, including

hypertension [22]. Troost et al. showed that nebivolol decreases systemic oxidative stress [23-25]. In addition, other studies reported that nebivolol has estrogen receptor agonistic properties [26, 27]. The role of estrogen in neuroprotection has been shown in multiple studies [28, 29]. A study showed that nebivolol acts as an estrogen receptor agonist in neuronal cell lines and has neuroprotective effects [30]. Another report found that nebivolol has a neuroprotective effect through its antioxidant activity [31]. The current study showed that nebivolol interacts with Keap1 by MolDock score -88.3 KJ/mol and can be used as an inhibitor of Nrf2 degradation. Also, molecular dynamics simulation indicates that Keap1-Nebivolol is a stable complex and reaches a steady state during simulation. Therefore, nebivolol can be used as a potential candidate for treating TBI.

Conclusion

These findings indicate that four molecules, ZINC435885061, ZINC469515563, ZINC119772060, and ZINC550706912, can suppress Nrf2 inhibitors such as Keap1, β-TrCP, and GSK-3, and they can be used as therapeutic compounds for TBI in the future. Also, Nebivolol, an FDA-approved drug, has neuroprotective effects and can be used for TBI treatment, but further studies, such as animal studies and clinical trials, are necessary.

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None.

Conflict of Interest Disclosures

The authors have no conflict of interests.

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None.

Authors' Contributions

All authors contributed in designing the study, data analysis and interpretation of data. Acquisition of data and drafting the first manuscript was done by HF and MAAE. Study supervision was done by FA in whole process and revising and approving the final manuscript was done by MAAE, FA, ST, HRR.

Ethical Statement

This research was approved by the ethical committee of Baqiyatallah University of Medical Sciences, Tehran, Iran, via code IR.BMSU.REC.1399.213.

Declaration of Generative AI and AI-assisted technologies

None.

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