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IN SILICO DESIGN AND PRECLINICAL EVALUATION OF NOVEL COMPOUNDS AS POTENTIAL NEUROPROTECTIVE AND ANTI-INFLAMMATORY AGENTS: A COMPREHENSIVE REVIEW

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Abstract
Neuroinflammation and neurodegeneration are characteristic of different disorders of the central nervous system, such as Alzheimer disease, Parkinson disease, and multiple sclerosis. The generation of new therapeutic agents to act on both neuroprotective and anti-inflammatory pathways has become an encouraging approach to the treatment of these debilitating conditions. This review has given an in-depth analysis of the present state of in silico drug design strategies and preclinical evaluation techniques used in the process of discovering dual-acting neuroprotective and anti-inflammatory molecules. We will talk about such methods of computations as molecular docking, pharmacophore modeling, QSAR analysis, and molecular dynamics simulations, as well as the preclinical evaluation plans that include in vitro and in vivo models. Recent discoveries in artificial intelligence and machine learning in drug discovery are also mentioned. Combination of computation and experiment methods has helped in speeding up the discovery of lead compounds and some of these have potentials of clinical translation.

Keywords: In silico, drug design, neuroprotection, anti-inflammation, molecular docking, preclinical, neuroinflammation

1. Introduction

The given review offers a clue on the contemporary issues and future trends in the area of the development of neuroprotective drugs.Neurodegenerative diseases are a significant global health concern, that is, affecting millions of people and causing serious socioeconomic consequences (1). These disorders have a complex pathophysiology which can be explained by a series of interconnected processes such as oxidative stress, protein aggregation, mitochondrial dysfunction, and chronic neuroinflammation (2,3). The conventional single-target methods of therapy have been

rather unsuccessful in the clinical trials, which has promoted more attention to the idea of multi-target therapy, which is capable of providing the simultaneous approach to neuroprotection and neuroinflammation (4).

Computational drug design has transformed the pharmaceutical industry, as it is now possible to screen large compound libraries in a short period and to make rational drugs designs (5). In silico approaches can provide low-cost alternatives to conventional high-throughput screening, which enables researchers to discover and optimize lead compounds and then subject them to costly experiments (6). A combination of these computational methods with a properly designed preclinical evaluation protocol gives a holistic paradigm of designing new neuroprotective and anti-inflammatory agents (7).

The central nervous system is especially challenging when it comes to drug delivery via the blood-brain barrier (BBB): hence, in the initial phases of drug discovery, computational prediction of BBB permeability plays a central role (8). In silico methods can be used to estimate ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties, which can assist in prioritizing compounds with good pharmacokinetic properties (9). This review will help give a general report on the existing computational methodologies and preclinical evaluation strategies that are being used in the discovery of dual-acting neuroprotective and anti-inflammatory agents.

2. Computational Approaches in Neuroprotective Drug Design

2.1 Molecular Docking and Virtual Screening

The technique of molecular docking has become a fundamental aspect of structure-based drug design as a method to predict the binding modes and binding affinities of small molecules and target proteins (10). Among the enzymes related to the oxidative stress response, inflammatory mediators and protein aggregation pathways, the following are the targets in terms of neuroprotective drug discovery (11). Screening campaigns using virtual screening technology have been used to identify new cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and nuclear factor-kB (NF-κB) signalling pathway inducers (12,13).

Docking predictions are accurate depending on a number of factors such as protein flexibility, interactions through water, and the choice of scoring functions (14). Enhanced successes of ensemble docking and induced-fit docking in the prediction of flexible targets like amyloid-beta aggregation inhibitors have been achieved recently (15). Neuroprotective potential scaffolds have been discovered by high-throughput virtual screening of large compound databases, such as ZINC, ChEMBL, and PubChem (16).

2.2 Pharmacophore Modeling

Pharmacophore modeling is the three dimensional structure of chemical properties necessary to the activity of the biological molecule (17). The method is especially useful in cases where the structural data of the protein of interest are scarce or when creating multi-target active compounds (18). There are structure-based and ligand-based pharmacophore models designed to inhibit a number of neuroprotective targets such as acetylcholinesterase, monoamine oxidase, and a-synuclein aggregation inhibitors (19,20).

The emerging generation of common-feature pharmacophore models facilitates the discovery of multi-target actives to nurture the complicated pathophysiology of neurodegenerative diseases (21). Pharmacophore modeling is enhanced by machine learning, and it has increased the accuracy of prediction during predictions and lowered the rate of false-positives in virtual screening campaigns (22).

2.3 Quantitative Structure-Activity Relationship (QSAR) Analysis

QSAR modeling provides mathematical correlations among the molecular descriptors and biological activities, whereby compound properties and lead structures can be predicted (23). The QSAR models have been used in the discovery of neuroprotectant drugs in antioxidant activity, BBB

permeability, and anti-inflammatory potency (24,25). Correlation of molecular descriptors such as topological, constitutional, geometrical, and electronic variables offer a comprehensive insight into structure-activity (26).
QSAR models have been boosted by machine learning algorithms such as random forest, support vector machines, and artificial neural networks which facilitate the predictive ability of these models (27). Consent QSAR modeling which involves combination of various algorithms has demonstrated greater reliability in forecasting neuroprotective profile and toxicity (28).

2.4 Molecular Dynamics Simulations

Molecular dynamics (MD) simulations are beneficial in detailing the interaction between proteins and their ligands, conformational change, and time stability in the binding process (29). This is because such simulations are fundamental in the study of the mechanism of action of neuroprotective compounds and maximizing their binding affinity (30). MD simulations have been widely applied in the inhibition of amyloid-betac aggregation, tau protein, and anti-inflammatory enzyme (31,32).
It is possible to predict binding free energies accurately with the use of free energy perturbation and thermodynamic integration techniques, which are used in optimization of leads (33). Very powerful acts of sampling, such as replica exchange molecular dynamics and/or metadynamics, have provided better exploratory power in the confined space of proteins and ligands (34).

3. Target Identification and Validation

3.1 Neuroinflammatory Targets

Neuroinflammation is a key driver in the development of neurodegenerative disease and, therefore, anti-inflammatory targets are of interest in therapeutic interventions (35). Some of the important targets are microglial activation markers, pro-inflammatory cytokines, and inflammatory signaling pathways (36). NF-κB signaling pathway that governs the expression of inflammatory genes have been widely used in the discovery of neuroprotective drugs (37).
The Toll-like receptors (TLRs) (especially TLR4) are key facilitators of neuroinflammation and can be used as potential therapeutic targets (38). Computational methods have discovered new neuroprotective TLR4 antagonists in preclinical systems (39). Opportunities to intervene may also be presented by the complement system, which is activated in the case of neuronal damage (40).

3.2 Oxidative Stress Targets

Neurodegenerative diseases are characterized by oxidative stress which is the consequence of the disproportion between the formation of the reactive oxygen and the antioxidant defense systems (41). Its main targets are antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, and one of the pathways, the nuclear factor erythroid 2-related factor 2 (Nrf2) (42,43).
Nrf2-Keap1 pathway controls the appearance of the antioxidant response factors and can be viewed as a prospective goal of neuroprotective intervention (44). The existence of new Nrf2 activators and Keap1 inhibitors with dual antioxidant and anti-inflammatory effects has been revealed through computational screening (45).

3.3 Protein Aggregation Targets

Protein misfolding and aggregation occurs in a variety of neurodegenerative diseases, including but not limited to: Alzheimer disease (amyloid-beta and tau), Parkinson disease (a-synuclein), and Huntington disease (huntingtin) (46). These aggregation processes provide therapeutic avenues to disease modification by targeting them (47).
Small molecules which can inhibit protein aggregation, promote disaggregation, and steer aggregation to non-toxic pathways have been described using in silico methods (48). Machine

learning models have been created to forecast the propensity of aggregation and determine possible inhibitors of aggregation (49).

4. In Silico ADMET Prediction

4.1 Blood-Brain Barrier Permeability

BBB is a significant drawback in CNS drug delivery, which limits the penetration of numerous potentially therapeutic compounds into the brain (50). BBB permeability prediction can be done using different computational models which include, but are not limited to: physicochemical property-based models, machine learning algorithms or molecular dynamics simulations (51).

Major molecular descriptors that determine the BBB permeability are molecular weight, lipophilicity, polar surface area and the capacity of hydrogen bonding (52). BBB permeability prediction models have been developed that have used in vitro BBB model data, such as parallel artificial membranes permeability experiments and cell-based models (53).

4.2 Toxicity Prediction

Timely detection of possible toxicity is important in the development of any drug and patient safety (54). The computational toxicology models are used to predict multiple toxicity endpoints, such as hepatotoxicity, cardiotoxicity, and neurotoxicity (55). Large databases of regulatory agency and pharmaceutical company toxicity data have been used to come up with structure-toxicity relationships models (56).

The toxicity predictions have been enhanced using machine learning techniques such as deep learning and ensemble methods (57). Combining the use of several toxicity endpoints with safety profiles will allow to assess risk and to better prioritize compounds (58).

4.3 Pharmacokinetic Properties

ADMET prediction involves absorption, distribution, metabolism, excretion and toxicity characteristics that help to define the fate of drugs in the biological systems (59). Pharmacokinetic prediction Computational models make use of a quantitative structure-property relationships and physiologically based pharmacokinetic modeling (60).

The oral bioavailability, plasma protein binding, metabolic stability, and clearance is one of the critical pharmacokinetic parameters of the neuroprotective drugs (61). Combining the pharmacodynamics models with pharmacokinetic modeling can determine the prediction of effective doses and dosing regimens (62).

5. Preclinical Evaluation Strategies

5.1 In Vitro Models

In vitro models allow the use of controlled experimental conditions to assess the neuroprotective and anti-inflammatory effects of new compounds (63). Primary neuronal cultures, immortalised cell lines and co-culture systems can be used to evaluate the effects of compounds on neuronal survival, oxidative stress, and inflammatory responses (64).

Model System	Application	Advantages	Limitations	Reference
Primary cortical neurons	Neuroprotection assays	High physiological relevance	Limited lifespan, variability	(65)
SH-SY5Y cells	Oxidative stress models	Standardized, reproducible	Limited neuronal characteristics	(66)
BV2 microglial cells	Neuroinflammation studies	Easy to culture, consistent	Mouse-derived, not human	(67)
Organotypic slice cultures	Complex tissue interactions	Maintains tissue architecture	Limited throughput	(68)
Blood-brain barrier models	Permeability studies	Predictive of in vivo transport	Variable barrier properties	(69)

Brain slices cultures Organotypic cultures provide the cellular interactions of the brain in vivo with the controlled experimental manipulation (70). The models are especially useful in the analysis of the impact of compounds on neuronal networks and glial-neuronal interactions (71). More physiologically relevant and with increased throughput, advanced in vitro models, such as microfluidic models and organ-on-chip models, are offered (72).

5.2 In Vivo Models

Neurodegeneration and Neuroinflammation animal model is necessary in the assessment of the therapeutic potential of new compounds in the complex biological systems (73). They are models that summarise the major pathological characteristics of human neurodegenerative diseases and allow to evaluate behavioural, histological, and biochemical endpoints (74).

Animal Model	Disease	Key Features	Behavioural Tests	Reference
APP/PS1 transgenic mice	Alzheimer's disease	Amyloid plaques, memory deficits	Morris water maze, Y-maze	(75)
MPTP-induced model	Parkinson's disease	Dopaminergic neuron loss	Rotarod, pole test	(76)
EAE model	Multiple sclerosis	Immune-mediated demyelination	Clinical scoring, rotarod	(77)
Stroke models (MCAO)	Ischemic injury	Focal brain ischemia	Neurological deficit scores	(78)
LPS injection	Neuroinflammation	Microglial activation	Open field, elevated plus maze	(79)

The choice of the suitable animal models is determined by the research question and the mechanism of action of the test compounds (80). Transgenic models offer understanding of chronic disease mechanisms whereas acute injury models offer the evaluation of neuroprotective outcome in reaction to particular harm (81). Multimodel systems integration improves the possibility of translation potential of preclinical results (82).

5.3 Biomarkers and Endpoints

The choice of suitable biomarkers and endpoints plays a very important role in measuring the effectiveness of the neuroprotective and anti-inflammatory compounds (83). Examples of biochemical indicators are oxidative stress parameters, inflammatory mediators, and protein aggregation (84). Non-invasive measurement of brain structure and functioning is made available through neuroimaging techniques (magnetic resonance imaging and positron emission tomography) (85).

Behavioral tests include the cognitive functioning, motor and neurological impairments (86). This combination of various endpoint measures gives a complete assessment of the efficacy of the compounds and assists in determining the most promising ones to be taken to clinical development (87).

6. Recent Advances and Case Studies

6.1 Artificial Intelligence in Drug Discovery

Neuroprotective drug discovery through the use of artificial intelligence (AI) and machine learning has led to improved and faster identification and optimization of lead compounds (88). The deep learning algorithms have demonstrated great success in the prediction of the properties of molecules, discovery of new drug-target interactions, and optimization of the structure of the compounds (89).

Variational autoencoders and generative adversarial networks are examples of the generative model that allows the de novo design of molecules with the intended properties (90). The techniques have been used to come up with new neuroprotective agents with high BBB permeability and low toxicity (91). Multi-objective drug design problems have been optimized through reinforcement learning algorithms on the basis of balancing efficacy, safety, and drug-likeness (92).

6.2 Successful Case Studies

A number of computational drug discovery packages have discovered neuroprotective compounds that have gone further to be developed into clinical (93). Virtual screening has identified the new g-secretase modulators, which are currently in clinical trials in the management of Alzheimer disease (94).

This has been made possible by structure-based drug design to provide selective phosphodiesterase enzyme inhibitors with neuroprotective effects (95). Computational and experimental methods have combined to enhance optimization of these compounds leading to the development of better potency and selectivity (96).

The multi-target approach of designing drugs resulted in the emergence of compounds that could be used as cholinesterase and antioxidants (97). Such compounds are encouraging in Alzheimer disease preclinical models and are a novel disease-modifying therapy (98).

6.3 Challenges in Translation

Even with the tremendous breakthroughs made in the computational drug discovery, it has been challenging to translate promising preclinical drugs to effective clinical therapies (99). The diseases of the neurodegenerative system, the species variance between the animal models and the human beings, and the impossibility of quantifying clinical endpoints contribute to the high failure rates in clinical trials (100).

Increased predictive animal models such as humanized models and patient-derived systems can be developed to enhance the translational performance of preclinical results (101). The combination of biomarkers and digital endpoints in clinical trials allows the detection of therapeutic effects to be more sensitive (102).

7. Current Challenges and Limitations

7.1 Computational Challenges

Although computational drug discovery has greatly improved, it is limited in a number of ways that affect the discovery of effective neuroprotective drug (103). Protein flexibility, weaknesses in scoring functions, and water molecules and metal ions treatment limit the accuracy of molecular docking (104). The accuracy of prediction needs to be improved by improving the sampling algorithms and the scoring functions (105).

Multi-target activity is a complex phenomenon that is difficult to predict because it requires specific algorithms (106). Machine learning models are expensive to develop using large and high-quality datasets, which are not always available on all the target classes (107). Model performance and generalizability are influenced by data quality (experimental variability and standardization problems) (108).

7.2 Preclinical Model Limitations

Neurodegeneration models on animals, though useful, have serious shortcomings in their ability to recapitulate the pathophysiology of human diseases (109). Differences in the metabolism, immune responses and disease progression in species could cause the translation of preclinical results to clinical success to be poor (110). Most of the animal models are acute, which may not be a good representation of chronic and progressive nature of human neurodegenerative diseases (111).

Extremes of non-standardized protocols and endpoints among the various laboratories make preclinical outcomes irregular and curtail meta-analyses (112). It must develop more predictive models such as human-based models and enhanced animal models to enhance translation (113).

7.3 Regulatory Considerations

The neuroprotective drug regulatory process is quite different because neurodegenerative diseases have a slow progressive nature and clinical endpoints are hard to quantify (114). The regulatory agencies need strong evidence of efficacy and safety, which might be difficult to illustrate with the existing biomarkers and clinical assessing tools (115).

Regulatory approval could also be made easy by the development of qualified biomarkers and digital endpoints that can give more sensitive indicators of therapeutic effects (116). The industry, academia and regulatory bodies should collaborate to formulate the right guidelines to be used in developing neuroprotective drugs (117).

8. Future Directions

8.1 Emerging Technologies

Quantum computing in the drug discovery process has the potential of addressing the computationally intractable complex problems in molecular computing (118). Quantum algorithms have the potential to allow more precise prediction of the molecular properties and protein-ligand interactions (119). Quantum machine learning methods could revolutionize the discovery of drugs because they can offer unprecedented computing power to solve complex optimization problems (120).

Structural biology, such as cryo-electron microscopy and nuclear magnetic resonance spectroscopy, is presenting new knowledge about protein structures and processes that can be applied to neurodegeneration (121). Such structural breakthroughs make computation modeling and drug designing endeavors more precise (122).

8.2 Personalized Medicine Approaches

Neuroprotection drugs can be designed based on the principles of precision medicine, and it is possible to create a therapy that corresponds to the particularities of a patient (123). Drug response and disease progression are genetically determined by the presence of polymorphisms of drug-metabolizing enzymes and disease susceptibility genes (124).

Combination of omics data, such as genomics, proteomics, and metabolomics, offers global comprehension of disease pathology and drug response (125). Molecular profile subtypes of patients can be recognized and used to predict their response to treatment using machine learning methods (126).

8.3 Systems-Based Approaches

The neurodegenerative diseases are complex and need systems-level solutions that involve the interactions of various biological pathways and processes (127). Drug discovery technologies based on networks are used to identify drugs that will interact with disease-relevant network modules, not with individual proteins (128).

Combining experimental data, at various scales, including the molecular, cellular, and system level, with computational models can offer a better understanding of the mechanism of disease and drug activity (129). Such solutions could result in the discovery of new treatment methods and combination therapies (130).

9. Conclusions

Combining computational and experimental methods has largely improved neuroprotective drug discovery as it has allowed discovering new compounds with neuroprotective and anti-inflammatory dual actions. Molecular docking, pharmacophore modeling, QSAR modeling and molecular

dynamics simulations are in silico techniques which offer potent tools of rational drug design and optimization of compounds. Computational drug discovery has also been improved by the use of artificial intelligence and machine learning to increase efficiency and accuracy.

Although a lot has been achieved, issues still exist in the process of translating promising candidates of preclinical experiments to effective clinical therapy. Neurodegenerative diseases have a high failure rate during clinical development due to their complexity, constraints of the existing animal models and regulatory issues. The future developments in computational techniques, experimental models and individualized medicine strategies are promising in conquering these challenges and coming up with the correct remedy to neurodegenerative illnesses.

The existing interdisciplinary approach of computational scientists, medicinal chemists, biologists, and clinicians is needed to enhance the sphere and apply scientific findings into therapeutic gains on the patient. The combination of new technologies, such as quantum computing, systems biology methods, can transform neuroprotective drugs discovery and result in breakthrough treatments of devastating neurodegenerative diseases.

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