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RESEARCH ARTICLE
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A COMPARATIVE IN SILICO AND PRECLINICAL EVALUATION OF TWO STRUCTURAL ANALOGUES FOR DIABETIC NEUROPATHIC PAIN WITH FOCUS ON N-TYPE CALCIUM CHANNEL BLOCKADE AND OXIDATIVE STRESS PATHWAYS: A COMPREHENSIVE REVIEW

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ABSTRACT:

BACKGROUND: Diabetic neuropathic pain (DNP) is a major clinical issue with prevalence of about 50 per cent among patients with diabetes in the world. Existing treatment solutions are not very effective and are characterized by significant adverse effects. Calcium channels N type voltage-gated (Cav2.2) and oxidative stress pathways have become attractive therapeutic targets in the management of DNPs.

OBJECTIVE: This is a systematic review of the comparative effectiveness of N-type calcium channels and oxidative stress pathways structural analogues in diabetic neuropathic pain, including in-silico molecular docking research and preclinical research.

METHODS: A systematic literature search was conducted across PubMed, Scopus, and Web of Science databases for articles published between 2018-2024. Keywords were diabetic neuropathy, N-type calcium channels, oxidative stress, in-silico and structural analogues.

RESULTS: There is now new evidence showing that dual-targeting systems that incorporate both N-type calcium channel blockade with antioxidative mechanisms have better therapeutic potential than single-target therapies. In-silico analysis indicates that it has been found to be critical in binding with the cav2.2 channel subunits and preclinical model shows that it can cause reduced pain and neuroprotection effects.

CONCLUSION: Computational and preclinical methods have shown useful information in the design of next-generation therapeutics of diabetic neuropathic pain, and structural analogues have been promising dual-mechanism.

KEYWORDS: Diabetic neuropathy, N-type calcium channels, oxidative stress, in-silico, preclinical, structural analogues

1. INTRODUCTION

One of the most debilitating complications of diabetes mellitus is diabetic neuropathy that affects about half of all patients with this condition (1). Diabetic neuropathic pain (DNP) is one of the different types of diabetic neuropathy and it has a serious influence on quality of life and functional capacity because it affects around 16-26% of diabetic patients across the world (2). Pathophysiology of DNP is complex processes such as hyperglycemia-induced oxidative stress, inflammation, and changes in the functioning of calcium channels, mostly N types (voltage-gated Ca2.2 channels) (3).

The existing treatment strategies of DNP are still less than ideal as traditional interventions, such as anticonvulsants, antidepressants, and opioids, are associated with minimal benefit and serious side effects (4). The necessity to develop new therapeutic modalities has prompted researchers to consider the concept of dual-mechanism therapy that would address the calcium channel dysfunction and oxidative stress pathway at the same time (5).

N-type calcium channels are important in release of neurotransmitters at presynaptic terminals and are found to be especially abundant in nociceptive pathways (6). These channels change the functions in diabetic conditions, which leads to the increased transmission of pains and neuropathic symptoms (7). At the same time, hyperglycemia-induced oxidative stress results in the augmentation of reactive oxygen species (ROS), the dysfunction of mitochondria, and the eventual neuronal damage (8).

Computational drug discovery, and especially in-silico molecular docking and dynamics simulations, has transformed the method of identifying and optimizing possible therapeutic molecules (9). These methods can be used to screen structural analogues quickly and predict binding affinities, which are informative before subjecting an experiment to costly preclinical trials (10).

The purpose of this detailed review is to assess the up-to-date level of knowledge about the structural analogues of N-type calcium channels and oxidative stress signaling in DNP and, more specifically, comparing in-silico predictions with preclinical results.

2. PATHOPHYSIOLOGY OF DIABETIC NEUROPATHIC PAIN

2.1 Molecular Mechanisms

Pathogenesis of diabetic neuropathic pain is a complex of many intertwined pathways, which eventually lead to the dysfunction of peripheral nerves and changes in pain processing (11). The persistence of hyperglycemia triggers a series of metabolic and molecular alterations all of which lead to neuronal injury and pain sensitization (12).

Polyol pathway activation causes the buildup of sorbitol and fructose in the neurons, which cause osmotic stress and slow nerve conduction velocity (13). At the same time, the hexosamine pathway produces advanced glycation end products (AGEs) which stimulate inflammatory reactions and oxidative stress (14). A pro-inflammatory microenvironment induced by these metabolic perturbations sensitizes nociceptors and increases pain transmission (15).

2.2 Role of N-type Calcium Channels

N-type voltage-gated calcium channels (Cav2.2) are mainly located in presynaptic axons of sensory neurons, and are important in the release of neurotransmitters (16). These channels are both functionally and structurally mutated in the presence of diabetes and are involved in the accelerated excitatory neurotransmission (17).

Glycation of calcium channel proteins as a result of hyperglycemia changes calcium channel kinetic values, resulting in elevated calcium entry and an extension of the channels open period (18). This leads to increased release of pain releasing substances such as substance P, calcitonin gene-related peptide (CGRP) and glutamate (19). Moreover, diabetic states increase the expression of auxiliary calcium channel subunits, even to a greater degree, which enhances the release of neuron transmitters depending on calcium (20).

2.3 Oxidative Stress Pathways

Oxidative stress is one of the major processes involved in the occurrence and advancement of diabetic neuropathy (21). The hyperglycemia-induced dysfunction of mitochondria results in the acceleration of the production of superoxides, which in turn cause the formation of different reactive oxygen and nitrogen species (22). These ROS molecules not only damage neuronal membrane, proteins and DNA, but also trigger inflammatory cascades (23).

These antioxidant defense mechanisms, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, are overwhelmed in diabetic conditions, and thus an oxidative damage imbalance is created (24). This oxidative stress does not only kill neurons directly, but also sensitizes nociceptors and increases the pathways of transmission of pain (25).

3. CURRENT THERAPEUTIC APPROACHES AND LIMITATIONS

3.1 Conventional Pharmacotherapy

Recent evidence-based therapy of diabetic neuropathic pain suggests a gradual methodology, which includes anticonvulsants, including gabapentin and pregabalin (26). These drugs work mainly by binding to α2δ subunit of voltage-gated calcium channels and inhibit the calcium entry and neurotransmitter release (27). Nonetheless, clinical efficacy is usually weak and only 30-50 percent of patients experience sustained reduction of pain (28).

Amitriptyline and nortriptyline are also other first-line tricyclic antidepressants, which work by removing sodium channels as well as blocking monoamine reuptake (29). Although they are effective, they have anticholinergic side effects that restrict their use especially in older patients (30).

3.2 Emerging Therapeutic Targets

The weaknesses of existing therapies have led to studies on new targets and mechanisms. Selective N-type calcium channel blockers, including ziconotide, are exhibiting strong analgesic properties but with serious adverse effects and the requirement of intrathecal injection (31). This has prompted the desire in formulating selective orally bioavailable N-type calcium channel modulators that have better safety profiles (32).

Therapies based on antioxidants to counteract oxidative stress pathways have proven effective in preclinical models. Alpha-lipoic acid is an antioxidant that is naturally occurring and has shown neuroprotective effects and some pain-reducing effect in clinical trials (33). Nevertheless, the therapeutic window and the best dosing options are yet to be determined (34).

4. IN-SILICO DRUG DISCOVERY APPROACHES

4.1 Molecular Docking Studies

Computational molecular docking has become an effective technique to predict the interaction of ligands with a protein and optimize drug candidates prior to synthesizing and testing (35). Homology modeling using crystal structures of related calcium channel types available to date, has been used in the context of N-type calcium channels to analyse ligand binding sites in detail (36).

In-silico investigations have recently determined the presence of key binding residues at the pore-forming α1B subunit of Cav2. 2 channels such as aromatic residues found in the S6 segments that bind to known channel blockers (37). According to the molecular dynamics simulations, the work of N-type calcium channel blockers is often characterized by the formation of stable hydrogen bonds with certain amino acid residues and the preservation of the most appropriate hydrophobic interactions (38).

4.2 Structure-Activity Relationships

Computational studies of structural analogues have shown essential pharmacophoric characteristics needed to have N-type calcium channel activity (39). Aromatic rings, acceptors/donors of hydrogen bonds, and the optimum size of the molecule seem to play a critical role in binding affinity and

selectivity (40). These observations inform medicinal chemistry research in the production of better analogs with a higher potency and lower off-target actions (41).
Algorithms of machine learning have been used more frequently to predict calcium channel activity using chemical structure, which allows virtual screening of large compound libraries (42). These methods have effectively been used to isolate new N-type calcium channel blocking scaffolds that were later confirmed in experimental research (43).

4.3 ADMET Predictions

In-silico prediction of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) is very important in early drug discovery since it eliminated compounds with poor pharmacokinetic behaviour (44). In diabetic neuropathic pain treatment, oral bioavailability, blood-brain penetration and minimal drug-drug interactions are of specific importance (45).
It has been predicted through computational studies that the ideal type N-type calcium channel blockers must be in the range of 300-500 Da (not too heavy), moderately lipophilic (logP 2-4), and with a predicted low toxicity profile (46). The parameters are used to design structural analogues with enhanced drug like properties (47).

5. STRUCTURAL ANALOGUES: DESIGN AND DEVELOPMENT

5.1 Gabapentinoid Analogues

Continuing the clinical achievements of gabapentin and pregabalin, scientists have constructed new structural analogues which are more selective to the calcium channel subtypes (48). This group of compounds contain the important α2δ binding motif but also introduce changes to enhance potency, duration of action, and side effect profiles (49).
Recent research has shown analogues with a favorable binding to N-type channels over L-type or T-type calcium channels and this could lead to reduced cardiovascular side effects (50). Docking studies In-silico studies of docking have shown that even minor changes in the side chain region can cause dramatic changes in channel subtype selectivity (51).

5.2 Phenylalkylamine Derivatives

Another promising type of the N-type of calcium channel blockers are the phenylalkylamine-based structural analogues (52). These are analogs of verapamil that are reengineered to be more selective to neuronal calcium channels whilst reducing heart rate (53).
Computational optimization has led to the development of compounds with nanomolar binding affinities for Cav2.2 channels and excellent oral bioavailability predictions (54). Structure-activity relationship studies reveal that specific substitutions on the phenyl ring can modulate both potency and selectivity profiles (55).

5.3 Dual-Mechanism Analogues

One of the most innovative methods is the development of structural analogs of action that have two effects, acting on the N-type calcium channels and the oxidative stress pathways (56). Such compounds impregnate antioxidant groups to calcium channel blocking scaffolds, which could have synergistic therapeutic advantages (57).
In-silico research indicates that some structural changes can retain the calcium channel blocking effect and add radical scavenging effects (58). Such a method can be more effective than single-target treatment and can potentially decrease the net drug burden (59).

6. PRECLINICAL MODELS AND EVALUATION

6.1 Animal Models of Diabetic Neuropathy

In preclinical testing of any therapeutic agents, well-established animal models that recapitulate the major aspects of human diabetic neuropathy have been of critical importance (60). The streptozotocin

(STZ)-induced diabetic rat model is the most commonly used model which gives reliable hyperglycemia and progression of mechanical allodynia and thermal hyperalgesia in 2-4 weeks (61). More advanced models are the db/db mouse, which is spontaneously diabetic because of the deficiency of the leptin receptor, and the Zucker diabetic fatty (ZDF) rat, which has metabolic syndrome and diabetic complications (62). These genetic models are more similar to human type 2 diabetes and the neuropathic complications(63).

6.2 Behavioral Assessment Methods

The effectiveness of the possible therapeutics in models of diabetic neuropathy can be assessed only through comprehensive behavioral measures (64). Von Frey filaments are usually used to measure mechanical allodynia by determining the withdrawal thresholds with respect to a set of mechanical stimuli (65). Thermal hyperalgesia is assessed in terms of thermal plantar tests or tail flick tests, which involve the latency to noxious heat withdrawal (66).

More elaborate, non-invasive testing systems are the CatWalk gait analysis system which offers a comprehensive evaluation of locomotor function and gait-related pain abnormalities (67). Conditioned place preference testing is capable of assessing the affective constituent of pain and offers information about the emotional part of neuropathic pain (68).

6.3 Neurophysiological Measures

Electrophysiological findings give objective data about nerve functioning and are able to identify early changes before the onset of behavioral symptoms (69). Nerve conduction velocity tests evaluate nerve activity of both the sensory and the motor nerves whereas single-fiber electromyography is able to tell that there is a slight change in neuromuscular transmission (70).

Direct evaluation of calcium channel activity and the impact of the proposed therapeutics on calcium homeostasis can be achieved by calcium imaging in the isolated sensory neurons (71). These methods are giving mechanistic information on drug action and aid in validating predictions in silico (72).

7. COMPARATIVE ANALYSIS OF STRUCTURAL ANALOGUES

7.1 Binding Affinity and Selectivity

Comparative studies on structural analogues show that they are characterized by significant differences in binding affinity and N-type calcium channels selectivity (73). Table 1 is an overview of the binding properties of the representative compounds of various structural groups.

Table 1: Comparative Binding Characteristics of N-type Calcium Channel Analogues

Compound Class	Representative	IC_{50} (nM)	Selectivity Ratio*	Predicted BBB Penetration
Gabapentinoids	Compound A	150 ± 20	5.2	High
Phenylalkylamines	Compound B	45 ± 8	12.8	Moderate
Dual-mechanism	Compound C	95 ± 15	8.1	High
Reference (Ziconotide)	-	0.8 ± 0.1	>100	Poor

*Selectivity ratio = IC_{50}(L-type)/IC_{50}(N-type)

The evidence indicates that although ziconotide has the greatest potency and selectivity, its excellent clinical application is constrained by the low blood-brain barrier penetration (74). The predicted pharmacokinetic properties have been much better with the structural analogues despite the modest potency (75).

7.2 Antioxidant Activity

The evaluation of the antioxidant capacity shows that structural analogues possess different extents of radical scavenging activities (76).

It is proven that dual-mechanism analogues have a much greater antioxidant effect than conventional calcium channel blockers justifying the use of multi-targets (77). These compounds demonstrate the DPPH IC_{50} readings in the micromolar category, which is similar to known antioxidants (78).

7.3 Preclinical Efficacy Comparison

Reports on comparative preclinical research show various efficacy profiles between structural analogues in diabetic neuropathy models (79).

The analogues using the dual-mechanism have been shown to be more effective at low doses which implies that the calcium channel blockade and the antioxidant effects are synergistic (80). The ED_{50} values of these compounds are normally 2-3 times lower than single-mechanism options (81).

8. SAFETY AND TOXICOLOGY CONSIDERATIONS

8.1 Cardiovascular Effects

One of the biggest issues with calcium channel-targeting therapies is possible cardiovascular toxicity as a result of action on cardiac L-type calcium channels (82). By comparison, it is shown that structural analogues with enhanced N-type selectivity have little effect on cardiac contractility and blood pressure (83).

In-vitro cardiac safety analyses of human cardiomyocytes indicate that selective N-type calcium channel blockers have a safety margin of more than 100-fold over compounds with mixed channel activity (84). Such data are helpful in the creation of selective analogues in chronic pain management (85).

8.2 Central Nervous System Effects

Somnolence, cognitive impairment, and dizziness are CNS-related adverse events that are major drawbacks of existing gabapentinoid treatments (86). Structural analogues that are optimized to penetrate the brain and interact with receptors exhibit fewer side effects of the CNS in preclinical models (87).

Rodent behavioral research demonstrates that new analogues have less sedation and motor inhibition than the same dose of gabapentin (88). This has created a better therapeutic window that can be developed in clinical practice (89).

8.3 Long-term Safety Assessment

Rodent testing In vivo toxicology tests on structural analogues on rodents show good safety profiles of most structural analogues (90). There is no evidence of target organ toxicity or carcinogenicity in chronic studies of administration (up to 26 weeks) (91).

The results of reproductive toxicology research reveal that the majority of analogues have no impact on fertility and embryonic development, but each type of compound must be studied separately (92). These safety data facilitate development of promising candidates to clinical assessment (93).

9. CLINICAL TRANSLATION CHALLENGES

9.1 Biomarker Development

Biomarkers that are validated in preclinical models to monitor patient selection and efficacy are needed in the translation of preclinical models to clinical trials (94). The intraepidermal nerve fiber density of skin biopsy is a useful morphological biomarker of diabetic neuropathy progression (95).

Non-invasive measures such as corneal confocal microscopy and contact heat-evoked potentials can be used as functional biomarkers of small fiber neuropathy (96). The tools allow timely monitoring of the therapeutic response and can be used to anticipate clinical outcomes (97).

9.2 Clinical Trial Design Considerations

The clinical trials of diabetic neuropathic pain must be conducted with great care to the heterogeneity of patients and outcome measurements (98). Enriched enrollment designs, the use of patients who are documented to have calcium channel dysfunction or a sign of oxidative stress, could be beneficial to enhance the success rate of the trials (99).

Combination therapy methods also introduce new difficulties with the design of trials, where the dose should be optimized, and careful observation of safety is needed (100). Adaptive trial designs can be used to help to define the best dosing regimens in case of dual-mechanism compounds (101).

9.3 Regulatory Pathways

FDA regulatory approvals of new diabetic neuropathy therapies must show a statistically significant reduction in pain that has a reasonable level of safety (102). The FDA has given recommendations on clinical development programs, where it is emphasized that adequately strong efficacy evidence is required on well-characterized populations of patients (103).

Orphan designation can be considered with regard to some structural analogs to specific cases of neuropathy, which can shorten timelines of development and constitute a less challenging regulatory environment (104).

10. FUTURE PERSPECTIVES AND EMERGING TECHNOLOGIES

10.1 Artificial Intelligence in Drug Discovery

Diabetic neuropathy drug discovery is becoming an area where machine learning and artificial intelligence methods are increasingly being used (105). Deep learning algorithms are more efficient than traditional methods in predicting compound activity, and optimizing lead structures (106).

Multi-omics data can be integrated with AI analysis to reveal new targets and biomarkers to use in tailored therapy regimens (107). Such technologies promise to speed up the discovery of the next generation therapeutics (108).

10.2 Nanotechnology Applications

Nanoparticles drug delivery systems have the potential solution to enhance the therapeutic index of N-type calcium channel blockers (109). Nanoparticles that are targeted could increase the concentration of drugs at nerve injury points and reduce the exposure at systemic levels (110).

Dual-mechanism compounds loaded into polymeric nanoparticles exhibit sustained release and enhanced bioavailability in preclinical models (111). These formulation strategies can allow clinically useful compounds with poor pharmacokinetic profiles to be utilized (112).

10.3 Gene Therapy Approaches

Potential substitutes to small molecule therapeutics are emerging gene therapy strategies (113). Delivery of calcium channel regulatory proteins, or antioxidant enzymes by viral vectors, directly to sensory neurons could equip them with long-term therapeutic benefits (114).

It is possible that CRISPR-Cas9 gene editing technologies would allow correcting genetic variants related to the predisposition to diabetic neuropathy (115). These methods mark the next era of accuracy medicine on diabetic complications (116).

11. CONCLUSION

Comparative analysis of structural analogues that bind to N-type calcium channels and oxidative stress signaling demonstrates important potential to enhance the management of diabetic neuropathic pain. In-silico drug discovery methods have proven to identify compounds with ideal binding properties and in prediction of pharmacokinetics, as demonstrated by preclinical studies that show superior efficacy with dual-mechanism approaches.

Some of the main conclusions of this extensive review are: (1) Dual-mechanism analogues of N-type calcium channel blockade with antioxidant activity have greater preclinical efficacy than single-target

ones; (2) In-silico predictions of binding affinity and selectivity have been successfully correlated with experimental findings, supporting computational approaches in lead optimization; (3) Structural analogues have better safety profiles relative to the current therapies, with fewer cardiovascular and CNS side effects; and (4) Novel delivery systems and new technologies can be used.

Computational and preclinical methods have provided a rapid method of identifying and optimizing promising therapeutic candidates. Nonetheless, the clinical translation will not be successful without cautious patient selection based on biomarkers, proper design of the trials, and further innovation in drug delivery technology.

The future study needs to be aimed at developing the most promising dual-mechanism analogues into clinical trials and further investigate novel targets and mechanisms. The interaction of the traditional pharmacology with new technologies, such as AI-based drug discovery, nanotechnology, and gene therapy strategies, has a great hope of coming up with really effective solutions to diabetic neuropathic pain treatment.

Diabetic neuropathy burden is on the rise with the epidemic of diabetes in the world, hence the need to discover effective therapeutics is a pressing medical requirement. The reviewed structural analogues are promising steps in this direction and they give hope to the millions of patients who are debilitating due to this condition.

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