Review Article

Animal Models of Neuropathic Pain

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ABSTRACT

This review paper presents the behavioural animal models that contribute to understanding and studying the neurological components of pain. Neuropathy is a complex condition that starts with the loss of sensory functions in the area of a limb, such as where someone had their leg amputated, and then continues to progress down the entire length of the leg. Researchers have begun to expand the understanding of how neuropathy occurs and the differentiation between the types of neuropathies; however, no one knows the definite cause of neuropathic pain. Researchers are using various types of animal models and biomarkers to investigate the mechanisms, pathology, pathophysiology, and pharmacology involved in the origin of neuropathic pain. This article will draw attention to the use of diabetes as an animal model for the study of neuropathy so that we can better explore and understand what is known and hypothesised to be the mechanisms producing neuropathy in both preclinical and clinical settings. Note that an ideal animal model of neuropathic pain must possess all the characteristics of a human pain state. The majority of models that are being used to investigate and test potential therapeutic agents for neuropathic pain are rodent models. The point of this paper will be on the animal models of diabetic neuropathic pain.

Keywords: Neuropathic behavioral models, diabetic neuropathic pain models.

INTRODUCTION

Clinical Features of Neuropathic Pain [NP]:

According to the International Association for the Study of Pain [IASP], neuropathic pain is elucidated as "pain resulting from a pathological process affecting the somatosensory nervous system." This was a substitute definition, from the previous definition, which interprets "pain that occurs in the central and peripheral nervous system that had either an initial incident, malfunction, or sometimes temporary changes with no evidence to support those abnormalities."[1] The actual occurrence of characteristic neuropathic pain has been set from population-based, longitudinal, or cross-sectional systematic studies of prevalence, being 7% to 10%. While neuropathic as well as non-neuropathic pain have a high rate of prevalence and influence, it's expected that further research will reveal neuropathic pain rated higher than non-neuropathic pain when expressed in line with severity.[2] There are numerous etiological conditions that can give rise to primary NP, which covers many peripheral conditions [e.g., chronic alcohol use, drug toxicity, diabetic mellitus (DM), cancer, herpes zoster infections] and traumatic injury to the nerves, in addition to cerebral events [e.g., cerebrovascular accident, multiple sclerosis, spinal cord injury] which affect the central system. The Patient with the NP reports different plus varying sensations of their pain. The most usual types comprise spontaneous pain, continuous pain, paroxysmal pain, and pain in response to stimuli. Pain in response to stimulus may involve allodynia [pain from non-painful stimuli that should not usually create pain —such as light touch, a gentle breeze] and hyperalgesia [pain from a minor stimulus produces severe discomfort and extreme pain]. A portion of patients experience a form of paraesthesia [sensations such as tingling, prickling, electrical shock, stabbing, and burning]. These unpleasant sensations remarkably cause morbidity and have been linked to reduced quality of life.[3] There can be both spontaneous (non-existing) or stimulus-induced neuropathic pain. Spontaneous neuropathic pain is very often related to a continuous burning feeling which is paroxysmal in nature and includes dysesthesias together with paresthesias.[4] According to epidemiological studies, a large number of individuals who suffer from NP do not get adequate care and treatment. While this may be due to lack of diagnostic precision or ineffectiveness of targeted drug therapy of NP, it can also be attributed to insufficient knowledge by physicians or clinicians about the use of effective medications in the management of neuropathy-associated chronic pain syndrome. Thus, it is vital to provide evidence-based recommendations on pharmaco-therapeutic management of NP.[5]

The advancement of efficacious mechanism-based therapies necessitates the utilisation of suitable animal models that accurately reflect the critical characteristics of peripheral neuropathy, alongside the implementation of relevant outcome measures pertinent to the disease's pathogenesis. Unfortunately, the literature is replete with unsuccessful clinical trials that depended on preclinical data derived from animal models that may not have been the most suitable or utilised outcome measures that are irrelevant to the underlying disease pathogenesis.[6]

Need for Preclinical Models:

There is a mismatch between the primary neuropathic pain assessment endpoints, and there is also clinical and

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preclinical evidence that shows how bidirectional research is serving to confirm new clinically relevant NP assessment endpoints. By customising both objective and subjective assessments, the translatability of novel medications for individuals experiencing NP is anticipated to enhance.

Finding biomarkers and target discovery:

Microneurography, neuroimaging, and electroencephalography [EEG] are presently the most promising ways to objectively measure neuropathic pain. These techniques can be performed on both preclinical species and humans, allowing for direct translation between species, thereby presenting a significant opportunity for the development of objective markers for NP.[7]

Mechanism elucidation:

Modern research successfully generated models of painful peripheral neuropathy in laboratory animals; evidence indicated that the animals suffer from both abnormal spontaneous pain and abnormal evoked pain, particularly allodynia and hyperalgesia. Experimental analysis of these models has elucidated potential pathophysiological mechanisms within both the peripheral and central nervous systems; it is probable that the neuropathic pain syndrome model arises from multiple mechanisms.[8]

Drug development and screening:

There are numerous criteria for evaluating recently developed models of pain, such as both behavioural methods of measuring pain and the end-points to evaluate pain. The ideal new animal models of pain should be designed as close as possible to the underlying disease process that causes pain, as well as the behavioural symptoms associated with the disease process, similar to humans. The emerging preclinical models should be able to use positive and negative control groups for distinguishing between preclinical models that are likely to yield false positives [i.e. positive control] vs. results with treatment known to lack effectiveness [i.e., negative control].[9]

Modelling Etiologies and Comorbidities:

Managing and addressing NP continues to be an issue for patients and medical professionals or physicians, particularly when comorbid with anxiety or depression. The novel treatment model may be critical since NP is often associated with severe chronic pain with few effective types of medication available. Researchers have reviewed and supported the use of Cannabidiol [CBD] and Beta-Caryophyllene [BCP] individually and together as viable alternative therapies for treatment while helping to reduce anxiety and depression symptoms.[10]

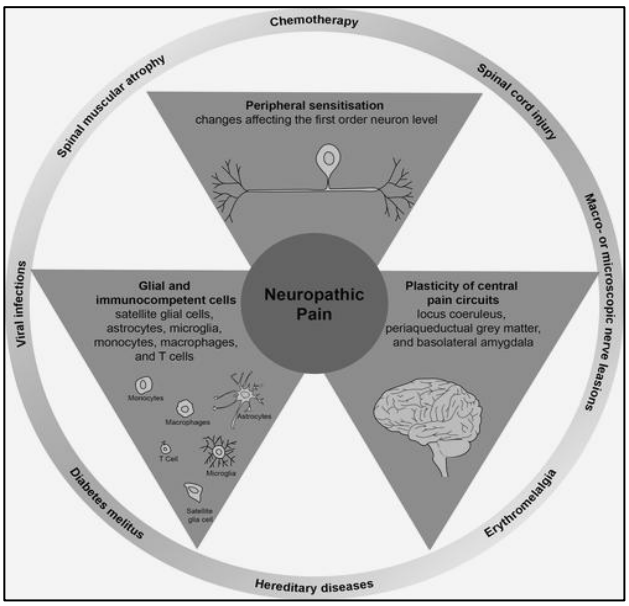
Scope of Review:

Rodent models continue to be important in the study of molecular, cellular, and neurophysiological processes associated with neuropathic pain. Overall, most animal research studies use models, which can be created through traumatic and surgical procedures or as a result of metabolic disorders, genetic defects, viral infection, chemicals that induce neurotoxicity and tumours.[11]

1. PATHOPHYSIOLOGICAL MECHANISM ASSOCIATED WITH NEUROPATHIC PAIN:

Neuropathic pain poses specific and distinctive challenges in diagnosis and treatment, as the pathophysiology is unrevealed and cannot be predicted based solely on the cause/etiology. There is insufficient specificity regarding the pathophysiology; thus, several theories proposing possible mechanisms of neuropathic pain, including peripheral and central nervous system sensitisation processes and neurogenic inflammation, have been broached.[12]

To abridge the various ways in which the mechanisms could potentially be linked and to display the overall framework of this review, several of the most frequently discussed pathways associated with NP are discussed and mentioned below according to their anatomical locations.



The schematic diagram presents a summary of mechanisms involved: peripheral sensitisation of first-order dorsal root ganglion neurons represented in the top triangle, alteration in the circuitry of central structures involved in the pain process represented in the right triangle, and modification in cross-talk and activity of glial, immunocompetent cells represented in the left triangle, as shown in Figure 1.

1.1 Peripheral Sensitization:

This is believed to constitute a significant mechanism in the development of NP. Many studies have demonstrated that first-order sensory DRG neuron [at the apex/top of the triangle] undergoes different molecular events leading to peripheral sensitisation. The enhanced function of voltage-gated ion channels in the plasma membrane of the DRG could represent an additional mechanism involved in this process.

1.2 Plasticity of Central Pain Circuits:

As indicated in Figure 1 [right triangle], plasticity of central pain circuits is a topic of discussion. The sensation of NP is

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an outcome of pathologic changes in the afferent pathway, and it is proposed that a rise in activity of noradrenergic neurons in the locus coeruleus, as well as other neurons and glial cells in the periaqueductal grey matter, contributes to progression, amplification, and persistence of NP. One target of the inhibition of pain transmission via the lateral spinothalamic tract is consequently compromised. Thus, the proposed pathway from the amygdala to the prefrontal cortex is a promising target for the design of neuropathic pain medications according to recent studies.

1.3 Glial and Immunocompetent Cells:

Neuroglia are a type of cell found in the central and peripheral nervous system that were previously thought to only serve as structural and nutritional support for nerves. Over time, however, the active role of neuroglia has become clearer, particularly the communication between them and neurons, which appears to be modified in individuals with NP. For example, the predominant glial cells responsible for framing neuropathic pain appear to change depending on the duration of pain. During the acute phase of pain, the activation of microglial cells is detected, while in the chronic phase, astrocyte cells get activated. Microglial cells are therefore believed to play an important role in the early stages of neuropathic pain and have been shown to become activated following peripheral nerve injury. While it is now known that glial cells have a wide variety of functions, including an important role in the regulation of pain response, other immune-competent cells must also be taken into consideration when trying to understand the occurrence of NP. For example, satellite glial cells help regulate the activity of nerve cell bodies in the DRG and have been shown to have altered coupling in those with chemotherapy-induced neuropathic pain and increased expression of the glial fibrillary acidic protein [GFAP]. In addition, macrophages, which are active in the PNS, have also been implicated in the development and maintenance of hyperalgesia during the pathophysiology of NP. Thus, it is important to recognise adequately to explain all the mechanisms responsible for signs and symptoms of NP; there are likely multiple mechanisms involved.[13]

2. CLASSIFICATION OF ANIMAL MODELS OF NEUROPATHIC PAIN:

2.1 Peripheral Nerve Injury [PNI] Models:

Typically, PNI commonly arises as a sequel of traumatic events. Recovery from PNI can frequently be inadequate, particularly in proximal injuries or events. For better understanding and promotion of peripheral nerve healing, researchers need a peripheral nerve injury model to evaluate, experiment, and identify therapies.[14]

Allodynia and hyperalgesia are common symptoms found among patients experiencing NP. Both symptoms occur in peripheral neuropathy and central pain-associated disorders at rates of 15-50% for patients who exhibit symptoms of NP.[15]

Example: Various animal models have been manipulated to study chronic neuropathic pain following peripheral nerve injury, including one of the most accepted models: the unilateral sciatic nerve chronic constriction injury [CCI] model developed by Bennett and Xie [1988]. The CCI is a very straightforward procedure, and results in long-term [i.e., at least one month after injury] development of substantial and consistent hypersensitivity to pain. Here, the procedure for performing the CCI and testing for hypersensitivity to pain is drafted. Prior to performing the CCI procedure, the rat is placed in an induction chamber for 5 minutes and then anaesthetised with 5% isoflurane in O_2 and subsequently 2% isoflurane in O_2 via a customised face mask; and monitored. Alternatively, anaesthetics that can be delivered to the animal could include inhaled halothane or intraperitoneal sodium pentobarbital. When the rat is on its chest/thorax, raise the left hind leg into a position in such a way that the left femur is perpendicular to the spine, and hold the left leg in that position with masking tape (on the shaved and sterilised foot). Make an incision in the skin for CCI, 3-4 mm below the left femur, parallel to the left femur, and dissect the skin away from the muscle surrounding the incision by carefully cutting through the connective tissue between the gluteus superficialis and biceps femoris muscles. Four Chromic gut ligatures are loosely tied with two knots around the sciatic nerve at 1 mm intervals until they occlude but do not completely stop epineural blood flow. The muscle will be sutured closed, and the skin will be stapled to close. After surgery, the animal will be allowed to recover for 24 hours before beginning pain hypersensitivity testing. To prepare for behavioural testing, the animal will be placed in the testing apparatus and given time to adapt. For days before starting the experiment, handlers should handle the animal to prepare it for handling during the experiment, which will help to minimise stress and potential for biting. Mechanical withdrawal threshold is set by using an electronic dynamic plantar Von Frey aesthesiometer to seek mechanical stimulation to both injured and uninjured hind paws. After mechanical withdrawal threshold testing, the rats will be housed in an elevated mesh-based testing cage for 15-30 minutes prior to testing to allow them to explore and groom in an organised manner. The maximum pressure applied to the paw prior to the withdrawal will be recorded as the mechanical withdrawal threshold. The form of the test uses a thermal plantar analgesic instrument to measure thermal withdrawal latency by placing the infrared source directly underneath the mid-plantar surface of the hindpaw through a glass surface. Following the infrared stimulation of the hindpaw, licking or shaking the hindpaw could be indicative of an animal demonstrating pain-related behaviours. Behavioural testing of the injured hindpaw by mechanical withdrawal threshold and thermal withdrawal latency both demonstrate a significant decrease in comparison to baseline and the uninjured hindpaw during the 2-12 days period following the CCI. Several other methods, such as the pinprick and cold allodynia assays, are used to assess pain hypersensitivity due to CCI. Additionally, scientific research employing the CCI model is advancing the understanding of the impact of neuro-immune systems on

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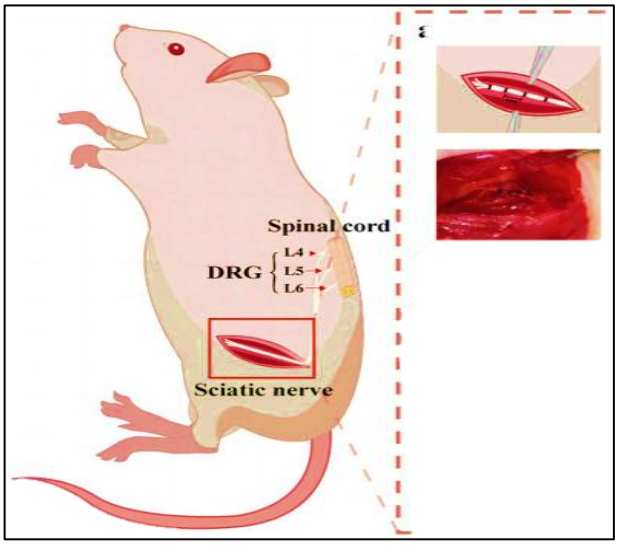
the initiation and treatment of NP. A limitation of this model is that there is substantial variation among groups of rats subjected to CCI, due to the variability associated with the tension of the knots used to create the constrictions with the sutures.[16]



Representation of peripheral nerve injury model — chronic constriction injury by placing four loose ligations around the sciatic nerve illustrated in Figure 2.[17]

2.2 Central Pain Models:

Central pain models have been designed mostly as a result of spinal cord injury. The features of traumatic, ischemic, compression, or crush-induced SCIs can include both dysesthesia and spontaneous and evoked pain.

Example: A central pain model like photosensitising SCI is that irradiating physical tissue generates a spinal cord injury using a photochemical reaction, specifically with the use of a photosensitising dye, erythrosine B, administered intravenously [IV] via tail vein. The subsequent surgical exposure of the vertebrae to a laser (argon ion) results in excitation and the production of the photochemical thrombus occluding the small blood vessels adjacent to the spinal cord that supply the nerves. It has been well documented that the nerve injury created through this process does not occur as a direct result of the heat produced; rather, the nerve injury occurs as a result of the photochemical reaction leading to the formation of thrombosis in very small blood vessels supplying blood to the nerves. The blood flow to the spinal cord has been shown to be dramatically reduced following the initiation of this photochemical process. There are also observable destructive lesions of the parenchymal tissue found in the area surrounding the spinal cord following the ischemic event resulting from this photochemical process. Numerous characteristics of neuropathic pain, such as cold allodynia, mechanical and autotomy, as well as hyperalgesia, are found in central pain models.[18]

2.3 Drug-Induced Neuropathic Models:

Peripheral neuropathy results from several causes and has a history of being a prevalent, painful condition. There is a range of medications like chemotherapeutic agents, antibiotics, cardiovascular agents, psychotropic substances, and anticonvulsants, as well as several others that have been found to be contributing factors in the development of drug-induced peripheral neuropathy resulting from iatrogenic injury. Chemotherapeutic agents have exhibited more consistently associated neuropathic events; however, many commonly recommended drugs by the majority of health care providers/doctors will have represented ADRs that include neuropathic involvement, such as statins.[19]

Example: Cisplatin-induced peripheral neuropathy [CIPN] is often recognised as a preclinical animal model for studying the effects of chemotherapy on the development of nerve injuries marked by both sensory as well as motor deficits associated with cisplatin chemotherapy. The main indications for the therapeutic use of cisplatin are for treatment of cancers of the ovaries, testicles, bladder and lungs. The accumulation of cisplatin in DRG neurons is the consequence of cell injury and cell death, which is mediated in part by increased output of reactive oxygen species leading to oxidative stress that damages the neuronal tissue. In addition, the inflammatory response mediates the interference of mitochondrial function, which results in the worsening and prolongation of nerve injury. The interest of this animal model is its correlative simplicity and flexibility with the dosing regimens. Investigators have been able to establish numerous routes of administration [e.g., single versus multiple dosing]. CIPN does not exactly mimic all the clinical features exhibited in patients receiving cisplatin chemotherapy; therefore, it is regarded as a linked drawback. Another obstacle is the variability of the onset and severity of neuropathy between animals and patients who have been treated with cisplatin. The level of severity of the neurotoxicity caused by cisplatin is closely associated with the total amount of administered drug—cumulative dosage. At low levels of dosage, patients may experience mild and reversible neuropathy, which can be indicated by symptoms such as tingling or loss of sensation in hands and feet, which may occur progressively. At intermediate levels, patients are likely to have symptoms that are much more severe than the low dosage category [e.g., persistent numbness, tingling sensations, or pain as part of their CIPN experience, with possibility of limited-to-no recovery]. Patients who are medicated with cisplatin at high cumulative dosages are likely to have a higher risk of experiencing very severe and irreversible neuropathy, which can manifest as severe pain, substantial loss of all types of sensations, and impaired motor functions. In addition to typically having a rapid onset of symptoms from taking the medication, patients also tend to have limited-to-no recovery of nerve function after terminating medications. An effective preclinical model of CIPN would consist of a sequence of carefully controlled studies designed to stimulate the neurological deficits experienced by individuals who are treated with cisplatin for the same conditions as the preclinical model.[20]

Research conducted on animal/ rodents for the development of cisplatin-induced neurotoxicity is reliant

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upon the methodology used for intraperitoneal [IP] injection. Primary adverse reactions associated with an IP injection are swelling in the peritoneal cavity and the genesis of ascites. In order to establish a chronic model of CIPN, an investigation is conducted to mimic the administration of multiple chemotherapies clinically, which involved choosing either 2mg/kg that is, given either 1 or 2 times per week, or 3 mg/kg, administered weekly for 4-5 weeks. Additionally, examiners are utilising various methods to assess mechanical hypersensitivity through mechanical pain threshold, hot water tail flick test [nociception], and motor deficits to estimate pain behaviour changes and to report/record success before starting the model. Primarily, investigators make use of Sprague Dawley and Wistar rat strains, although other studies have shown that males have different behaviours when compared to females during the development of CIPN. Notably, males lost much greater weight than females due to stronger jerks caused by chemotherapeutic agents, a much longer time delay before their paws shrink in response to heat, and decreased nerve conduction velocity compared to females.[21]

Table 1: Overview of classification of animal models of neuropathic pain.

	Name of Model	Principle Involved	Animal Species
Peripheral Nerve Injury Model			
A	Axotomy	Complete transection of sciatic nerve	Rats
B	Chronic constriction injury	Four loose ligatures around the sciatic nerve	Rats, mice
C	Partial sciatic nerve ligation (Seltzer Model)	Tight ligation of one-third to half of the sciatic nerve	Rats, mice
D	Spinal nerve ligation	Spinal nerve ligation (i) Tight ligation of L5 and L6 spinal nerves (ii) Tight ligation of L7 spinal nerve	Rats, Macaca fascicularis
E	Spared nerve injury	Axotomy of tibial and common peroneal nerves	Rats, mice
Central Pain Model			
A	Weight-drop or contusive spinal cord injury	Dropping a weight over the exposed spinal cord	Rats, mice
B	Excitotoxic spinal cord injury	Intraspinal injection of excitatory amino acids	Rats, mice
C	Photochemical spinal cord injury	Thrombosis in blood vessels supplying the spinal cord by photosensitizing dye and laser	Rats
D	Spinal hemisection	Laminectomy of T11-T12 segment	Rats
Drug-induced Neuropathy Models			
A	Anti-cancer agents [vincristine, cisplatin, oxaliplatin, paclitaxel]	Direct injury of drugs to the nerves of the peripheral nervous system	Rats, mice and guinea pigs
B	Anti-HIV agents [2,3-dideoxycytidine, didanosine]	Direct injury of drugs to the nerves of the peripheral nervous system	Rabbits, rats
Disease-induced Neuropathy Models			
A	Diabetes-induced neuropathy	Persistent hyperglycemia-induced changes in the nerves	Rats, mice
B	Cancer pain models (a) Femur, calcaneus, tibial, and humerus bone cancer pain (b) Neuropathic cancer pain (c) Skin cancer pain of hind paw	 Inoculation of cancerous cells into respective bones Growing a tumour in the vicinity of the sciatic nerve Injection of melanoma cells	 Rats, mice Mice Mice
C	HIV-induced neuropathy	Delivery of HIV-1 protein gp120 to the sciatic nerve	Rats
D	Post-herpetic neuralgia (a) Herpes Simplex Virus	Depletion of capsaicin-sensitivity	Rats
Miscellaneous Models			
A	Chronic ethanol consumption/withdrawal	Adm. of ethanol over extended period around 70 days	Rats
B	Pyridoxine-induction	Adm. of high doses of pyridoxine for a long period	Dogs, rats
C	Trigeminal Neuralgia	Compression and constriction injury to the infraorbital nerve	Rats
D	Acrylamide-induction	Adm. of acrylamide for prolong time period	Dogs, rats

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2.4 Disease-Induced Neuropathy Models:

Three of the most widely researched disease models that have a crucial influence on public health include diabetic peripheral neuropathy, chemotherapy-induced peripheral neuropathy, and human immunodeficiency virus [HIV]-associated sensory neuropathies.

Example: HIV infection and subsequent therapy can result in a variety of peripheral neuropathies, depending on the exact stage of the disease and specific antiretroviral drug regimen employed. The clinical presentation of distal symmetric polyneuropathy associated with HIV infection resembles that of toxic polyneuropathy as an outcome of a specific type of antiretroviral drug treatment; therefore, distal symmetrical polyneuropathy secondary to HIV is collectively referred to as HIV- associated sensory neuropathies [HIV-SN]. Many researchers have been focusing their attention on exploring the neuropathic pain, which is a defining feature of many patients with HIV-SN. Scientists have focused on understanding the neuropathic behaviour using a research model whereby Wistar rats are administered IP zalcitabine [3 times/week for 3 weeks] injections, and investigated both evoked pain [mechanical hyperalgesia] and non-evoked pain behaviours [anxiety-like behavioural outcome—presumably due to spontaneous pain]. The administration of gabapentin and morphine reduced anxiety-like behaviours. As a result, the model was useful for evaluating the potential pharmacotherapeutic efficacy of commonly prescribed medications [such as gabapentin, morphine, and cannabinoids] used to alleviate neuropathic pain.[6]

The herpes virus is another virus that has been associated with the occurrence of latent neuropathic infection within the host, and subsequent recurrence of the disease via the reactivation process. Currently, there are various types of known herpes viruses [e.g., neurotropic herpes simplex virus type 1(HSV-1)] which infect both the central and peripheral nervous system. According to preliminary literature recently published, the consequences of HSV-1 infection include not only the development of encephalitis but also the possibility of causing peripheral neuropathy. There have been isolated cases described with patients showing clinical signs of both HSV infection and peripheral neurological issues [e.g., muscle weakness] with the exception of developing facial paralysis.[22]

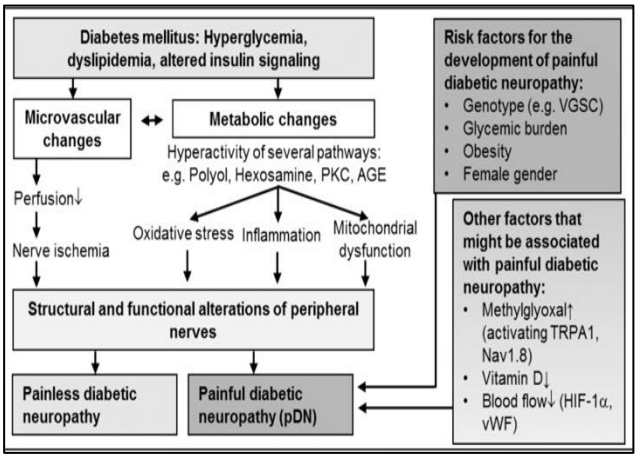
2.5 Miscellaneous Models:

Acrylamide-induced neuropathy refers to the effects of acrylamide, a neurotoxin that affects both the central as well as the peripheral nervous system. The effects of exposure to acrylamide include both large and small nerve fibre damage by acrylamide; however, the pathways of exposure may be different. The pathobiology of the acrylamide-induced neuropathy is unique, and the phenomenon known as “dying back” [the early degradation of distal axons]. Each stage in the development of acrylamide-induced neuropathy can be differentiated by massive oxidative stress as one of several neurotoxic mechanisms. Repeated and low-level doses of acrylamide [35-140mg/kg total dose], when given to preclinical models, resulted in the development of mechanical allodynia and thermal hyperalgesia, without affecting motor capacity. Antioxidants may be effective at reducing the accumulation of reactive oxygen species [ROS] in in vitro models and restoring the toxic effects of acrylamide in animal models.[23]

3. REVIEW FOCUSES ON ANIMAL MODELS OF DIABETIC NEUROPATHIC PAIN:

Diabetes mellitus [DM] is an emerging pandemic with a rapidly increasing prevalence due to changing lifestyles. An increase in DM leads to a similar or even greater increase in late complications of DM, e.g., neuropathy.[24] According to the Global Diabetes Epidemiology site, neuropathies are the most common late complication of diabetes [also known as diabetic neuropathies, since they are multifactorial causes of neuropathy in DM]. Diabetic neuropathies result in a loss of sensory function in the lower extremities with pain and significant morbidity.[25]

The estimated lifetime prevalence of diabetic peripheral neuropathy [DPN] is >50%, and 15%-25% of patients with diabetes suffer from neuropathic pain [also known as “painful DPN”]. Appropriate management of painful DPN is vital for improving quality of life due to its negative impact on sleep, anxiety, and depression. The foundation of the treatment approach for managing painful DPN includes controlling hyperglycemia and other modifiable risk factors; however, these approaches may not be enough to prevent and treat DPN. As there are no currently available disease-modifying medications for the treatment of DPN, it is important that pain be managed as a symptom of painful DPN.[26]



Mechanism relevance to diabetic neuropathic pain is shown in Figure 3.[27]

There are several approaches to inducing diabetes in rats and mice using genetic, dietary, and chemical means, and a number of established models of diabetic neuropathy can appropriately represent both type1 and type 2 diabetes. This model allows for studying possible pathophysiological mechanisms of diabetes and developing potential pharmacological therapies to alleviate established pain or prevent pain from occurring.[28] Much of what we know

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about how diabetes causes problems with nerves comes from experiments that have been performed on rats and mice that have diabetes. The most common type of diabetes model used in research is one that has been made to have diabetes by injecting them with a drug called streptozotocin or by feeding them a diet high in fat, or some combination of a toxic chemical [like streptozotocin] and a diet high in fat. There are also other ways to obtain diabetic rats and mice that could be used for research other than the streptozotocin and high-fat diet models; these include genetically distorted animals that develop diabetes spontaneously [e.g., Zucker diabetic fatty rats; type 1 insulinopenic BB/Wor rats; type 2 hyperinsulinemic diabetic BBZDR/Wor rats], non-obese diabetic mice, Akita mice. A variety of methods have been used to determine how diabetic animals respond to the environment [e.g., pain sensitivity to heat and mechanical stimulation; tactile allodynia; etc, and spontaneous nociceptive behaviours induced by formalin administration]. The following review will discuss a selected number of animal models identified as valuable in understanding the biological basis for neuropathic pain associated with diabetes mellitus.[29]

3.1 Induced Models:

There are several different versions of animal models, including type 1 and type 2 diabetes animal models, to study neuropathy in rodents. Animal models that are used to study diabetic neuropathy can be classified into two main categories, induced and genetic models. The induced models can be further subdivided into two different categories based on how they are created: drug-induced diabetic neuropathy models and diet-induced diabetic neuropathy models.[30]

3.1.1 Drug-Induced Model:

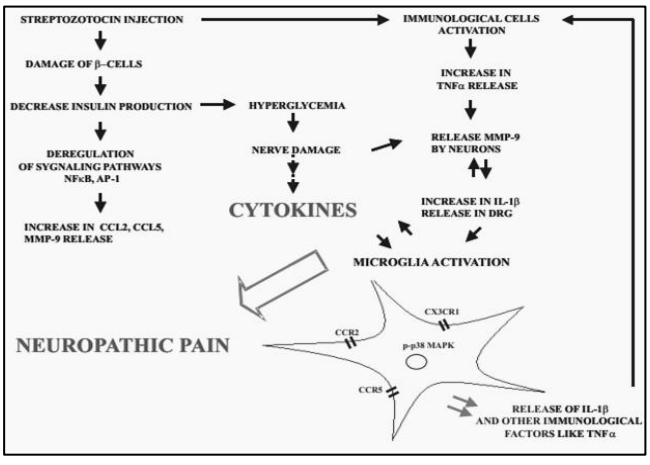
A variety of pharmaceutical agents or chemical can be utilised as agents for inducing diabetes in rodent-bases animal models including streptozotocin [STZ], alloxan, vactor, dithizone, 8-hydroxyquinolone, and gold thioglucose; however, the most widely utilized chemical agent to create animal models for experimentation with type 1 type 2 diabetes is STZ, as one of the leading chemical/drugs used for inducing the formation of diabetes.

A. Streptozotocin-Induced Model:

In order to chemically induce an animal model of type 1 diabetes, one can administer STZ via either a high single or low multiple doses to rats, e.g., a single injection of 15mg/kg IV or 20mg/kg SC on five consecutive days. The chemical streptozotocin [also referred to interchangeably as "streptozocin"], which has the chemical structure of 2-deoxy-2(([methyl(nitroso)amino]carbonyl)amino)-(α *and* β)- D-glucopyranose, was discovered to occur naturally by the organism Streptomyces achromogenes and was identified as an antibiotic in 1959. In 1963, the chemical's deleterious effect on the pancreatic β-cell was described by Rakieten et al. The molecular formula of STZ is $C_6H_{15}N_3O_7$ with an approximate molecular weight of 265g/mole, having two sections: the glucopyranosyl moiety, which assists with the entry of STZ into the pancreatic β-cells via the glucose transporter [GLUT2], and the nitrosourea moiety, which is responsible for the destruction of the pancreatic β-cells.[31]

The development of STZ-induced diabetes is relatively easy due to the fact that it can be induced by a single injection [IV or IP]. Consequently, this model has been used extensively in research to investigate the mechanisms underlying diabetic neuropathic pain and to assess the efficacy of potential treatments. There are various doses of STZ [35-200 mg/kg] that have been evaluated for their ability to cause β-cell failure across various species, including rats and mice. The susceptibility of an animal to developing diabetes from STZ depends on the strain, species, and age of the animal, so there is a range of dosages that are effective in developing diabetes in an animal. When administered at higher doses of STZ into rats, the resultant type of diabetes produced is chemically similar to that type of diabetes seen in humans with insulin-dependent type 1 diabetes; i.e., the manifestation of symptoms of hyperglycemia and decreased glucose stimulation of insulin secretion occurs within 72 hrs following injection of STZ.[29]

A random sample of female Sprague-Dawley rats with a weight of 175- 200 g [7±1 week old] will be randomly assigned to either the saline control or experimental treatment group. 16 hours after the last injection, the rats will be weighed and injected with a single dose of STZ [50 mg/kg, IP] for the purpose of producing diabetic conditions. Due to the instability of STZ, fresh solutions will be made on the day of administration. The rats will have free access to food and water, and will be monitored throughout days 1,5,15,29, 5, 15, and 29. Blood glucose will be monitored using a glucometer, and tail tip blood samples will be collected from the rats. Body weights for the saline control and diabetic rats will also be measured throughout the entire study protocol. The presence of early indicators of diabetic neuropathy has been observed in STZ-induced diabetic rats, as evidenced by the development of allodynia; decreased nerve conduction velocity; and axonal dystrophy, as determined by electron microscope analyses of the rat's nerve axon structure.[32]



A diagram of the cytokine network in the pathogenesis of STZ-induced neuropathy is illustrated in Figure 4.[33]

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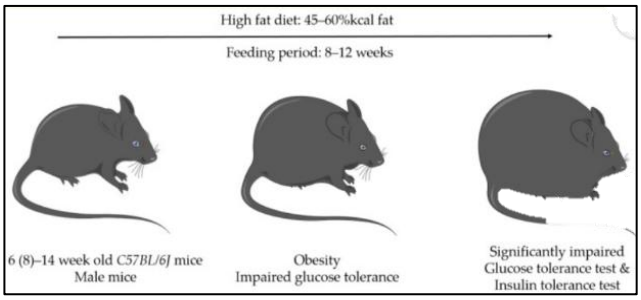
3.1.2 Diet-Induced Model:

About 30% of patients with prediabetes and around 60% of T2D patients develop peripheral neuropathy as a serious complication. It appears that metabolic syndrome is a commonly recognised, but often not attributed, independent risk factor for developing neuropathy. To date, very little basic and clinical research has been done to explore how peripheral neuropathy can occur after nerve damage; the use of animal models—diet-induced models that replicate human metabolic disorders [obesity, prediabetes, T2D and peripheral neuropathy]- has all served as/ verifiable models useful for understanding and exploring pathophysiological mechanisms.[34]



Depiction of high-fat-diet-induced mouse model in Figure 5.[35]

A. High-Fat-Diet [HFD] Fed Mice:

T2D diabetic neuropathic pain has been successfully established in adult experimental animals using methods based on human metabolic syndrome and nutritional induction using overfeeding of HFD. HFD is defined as one that has a 24% [by weight] of fat from a mix of soyabean oil and lard, a 24% [by weight] of protein and a 41% [by weight] of carbohydrates provided over a period of 12 weeks; using the C57BL/6 mouse strain in research studies examining resulted in the development of the same signs and symptoms of both prediabetes and neuropathy in mice. Phenotyping measures/parameters include, but are not limited to, decreased sensory nerve conduction velocity [SNCV], reduced density of intraperitoneal nerve fibres [IENF] and thermal hypoalgesia. There are a number of different factors that can affect the degree of neuropathy in these experimental models; differences in specific methods of neuropathy phenotyping measurements, differences in sex and/or age of animal tested, as well as duration of HFD feeding period and/or source and percentage of fat in HFD fed by animals have been previously reported. According to the Jackson Laboratory, male mice are the preferred models for dietary/nutrition induced T2D; this is thought to be due to variability in the two genders of animals based on their response to pain. Diet-induced [HFD] T2D animal models do not provide suitable models for use in the study of prediabetic and/or obesity-related neuropathies in these donor mice.[36] The C57BL/6 mouse strain has been most commonly used to study the physiological mechanisms associated with T2D as well as response to intervention studies. Mice fed high-fat diets (60% caloric intake from fat diets) develop diet-induced obesity and diabetic neuropathy at around the same rate [at least 8 weeks after the feeding]. The disadvantage of using this model is that it may take a considerable amount of time before neuropathic changes become evident, and monitoring caloric intake on a daily basis can be difficult. Overall, these rodent models make them very effective for investigating insulin resistance and pre-diabetes-related neuropathic changes.[37]

B. HFD + STZ-Induced Models:

It has been generally believed that T2D is primarily the result of a combination of genetic predisposition and unhealthy lifestyle habits, including consuming an “energy dense” or westernised diet. Thus, the researchers have relied on diet-induced rodent models of T2D that are subjected to chemical stressors [HFD] or drug-induced stressors [STZ] to closely mimic the pathological progression of diabetic complications in humans.[38] In 2000, a study by Reed et al. was proposed, which mentioned that rodents could be diagnosed with β-cell dysfunction by combining HFD with a single injection of STZ. Eight-week-old male Wistar rats were taken and kept in pairs in cages under a 12:12-hour light-dark cycle at 22°C. Among them, six rats were fed a high-fat and high-fructose [HF/HF] diet containing 25.7 wt.% lard and 46.5wt.% fructose; both groups received free access to water for a total of 56 weeks. Then, all juvenile HF/HF rats received an IP injection of a low dose of [25mg/kg]. Control animals received an equivalent volume of sodium citrate buffer [2% volume]. STZ was injected four times at equal intervals [every six weeks] from the initial injection to produce frank hyperglycemia as a result of chronic stress applied to the Langerhans islets. The weight of the HF/HF/STZ rats was recorded once per week, and the postprandial glycemia of all subjects was evaluated repeatedly over the course of the investigation. Results demonstrated that HF/HF/STZ rats developed robust tactile allodynia. In addition, electron microscopy revealed that many of the myelinated axons of the sciatic nerves of these rats were severely damaged compared to the control group. Damage to these axons in HF/HF/STZ rats also indicated that mitochondrial abnormalities were present, based on atypical mitochondria in the axons.[39]

3.2 Genetic Models:

The genetic models used in the studies of diabetic neuropathy for T2D use genetically modified animals, which include Goto Kakizaki rats, Zucker Diabetic Fatty [ZDF] rats, BBZDR/Wor rats, Otsuka Long-Evans Tokushima Fatty [OLETF] rats, Nagoya Shibata Yasuda mice, and db/db mice.[30]

3.2.1 The Zucker Diabetic Fatty Rat Model:

Male ZDF [fa/fa] rats are derived from the inbreeding of hyperglycaemic Zucker obese rats. These leptin receptor-mutated rats have high circulating levels of leptin. The onset of noticeable hyperglycaemia [400-500mg/dL] in male ZDF rats occurs between 7-10 weeks of age and can be maintained up to 6 months after the onset of diabetes. Hyperlipidaemia, insulin resistance and hyperinsulinemia may develop in these animals at 7 weeks of age; their

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circulating insulin levels will reach approximately 8 times that of the circulating insulin levels in lean controls before decreasing over time due to β-cells exhaustion. The weight gain during the first 6 months of life for male ZDF rats is greater than that observed in lean controls [up to 500 g]; therefore, this weight gain will be slightly reduced.[40]

3.2.2 The Bio-Breeding Zucker Diabetic Fatty Rat [BBZDR]/Wor Type 2 Diabetes Rat Model:

The BBZDR/Wor rat model is associated with T2D, which has been developed as an inbred rat colony model, created using classical breeding methods from the introgression of the dysfunctional leptin receptor gene [*Lepr*^{fa}] from the insulin-resistant ZDF rats. Male BBZDR/Wor rats with diabetes will embody the fatty mutation due to being homozygous for that recessive mutation, and carry almost identical genetic background sequences to the original BB rat strain. The BBZDR/Wor rats will develop diabetes-related complications due to consistently elevated blood sugar levels similar to patients with T2D, which would also include inflammation and nerve injury at the site of impeded blood flow.[41] Male BBZDR/Wor rats spontaneously develop diabetes at 70-80 days of age and are generally obese prior to the development of T2D. The development of diabetes is accompanied by the development of insulin resistance, hyperinsulinemia, hyperglycaemia, hypercholesterolemia, dyslipidaemia, and hypertension, which tends to occur later in the progression of T2D. Within 4 weeks of developing diabetes, conduction velocity deficits, thermal hyperalgesia and the degree of sural nerve fibre loss will occur, along with other qualitative differences to the sural nerve injury observed in Type 1 BB/Wor rats. The key or major advantage of this model is that they develop hyperglycemia without intervention and will remain hyperglycemic for a prolonged period depending on food intake. High cost and the severity of the illness oftentimes necessitate the need for insulin supplement use in these rats.[42]

4. ASSESSMENT METHODS IN ANIMAL MODELS OF NEUROPATHIC PAIN:

This section provides an overview of various methods used to identify neuropathic pain in rodent models, including both stimulus-evoked and non-stimulus-evoked approaches. It is challenging to assess neuropathic pain in animal models. Historically, most reports of neuropathic pain recorded in the literature come from subjective observational studies of response to stimulus-evoked pain behaviours, including withdrawal from noxious stimuli, licking or shaking of body parts stimulated by noxious stimuli, and vocalisation in response to noxious stimuli. The Von Frey test, for example, is commonly performed to assess mechanical allodynia in rodents. Similarly, various tests, such as the hot plate, Hargreaves and acetone, can be used to assess thermal sensitivity. Nociceptive behaviour can be assessed by following chemical stimulation [e.g., capsaicin or formalin] by intraplantar injection. In addition, there are several alternative tests for estimating neuropathic pain with regard to cold hyperalgesia and allodynia, which include the free choice thermal preference test, conditioned place preference test, and place escape avoidance test, as well as reward-based assessments of ongoing pain in rats and mice using a conditioned place preference approach. Finally, methods based solely on observation can also be used to detect spontaneous neuropathic pain. Rodents' facial expression is a source of insight into well- being through assessing the intensity of orbital tightening, cheek flattening and nose changes, ear position and orientation/shape, and whisker position or length. Grimace scales have been published to help monitor and quantify non-stimulus-evoked nociception and are useful in assessing responsiveness to pain in rodents. In addition to the previous methods that may be analysed, the burrowing behaviour, the weight bearing, and gait of the animal can also contribute to and confirm the nociceptive response collected data. However, interpreting the altered behaviour of the rodent requires caution, as they reflect discomfort [e.g., pain], but need not necessarily reflect pain alone. Furthermore, the aforementioned methods are not appropriate for use with every model. For example, the species being utilised for an injury model when limb nerves have been manipulated, and weight bearing and gait will often be impacted regardless of whether the animal is also in pain. However, through close monitoring of the rodents, non-stimulus-evoked nociception may still be identified, which is a critical characteristic of NP in human patients. This particular method of assessing stimulus-evoked pain in animals has one significant advantage over the other methods described above [i.e., high cost], but the downside is that close monitoring of animals will be time-consuming, requiring specialized training of the experimenter, and most notably need for the development of a standardized biomarker that would facilitate the functional in-vivo evaluation of NP in rodent models and also provide an adequate readout of in-vitro models going forward.[13]

4.1 Biomarker Assessment:

The identification of a suitable and widely applicable biomarker is critical to determine the stage of progression, progress, and new therapeutic options in diabetic neuropathy, with particular emphasis being placed on identifying biomarkers that correlate with both pathophysiological and clinical pain-related behaviour. Numerous functional, behavioural, structural, and molecular biomarkers of diabetic neuropathy have been identified in rodent models.

4.1.1 Behavioural Biomarkers:

A. Neuropathic Pain

Assessment of NP in diabetic rodent models can be carried out through multiple means, including thermal [tail immersion, hot plate], mechanical [paw pressure, Von Frey hair] or chemically-induced [exposure to high/low concentrations of formalin (0.5-5%)/(0.2%) administration] methods.

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B. Motor Coordination:

Diabetic neuropathy due to large fibre causes dramatic overall impairments in motor and sensory ability. A beam-work apparatus was shown to be a sensitive measure of this change. The number of slips by hindpaw of mice during crossing over the beam-walk apparatus [the measured parameter of sensorimotor function] was counted. After 10-weeks of hyperglycemia, the diabetic mice demonstrated significantly greater numbers of hindpaw slips than did nondiabetic mice, which is a marker of diabetes-induced damage to large fibres.

4.1.2 Functional Biomarkers:

A. Slow Sciatic Nerve Conduction Velocity [SNCV] and Slow Motor Nerve Conduction Velocity [SMNV]:

A Nerve Conduction Velocity [NCV] test is an electrical test that is used to evaluate the conduction of a nerve impulse along a nerve and to detect abnormalities of conduction and/or injury to the nerve. Diabetic rodents are demonstrated to have reduced SNCV and MNCV.

B. Reduced Nerve Blood Flow [NBF]:

Decreased NBF was noticed in diabetic rats after 8-weeks of the onset of diabetes. Decreased NBF with resultant nerve hypoxia may be one of the mechanisms responsible for the decreased NCV associated with diabetes. Several techniques have been used to assess NBF, including H_2-polarography, 14C-iodoantipyrine and laser Doppler velocimetry.

C. Delayed Gastric Emptying, Intestinal and Colon Transit:

These are markers of gastroparesis in diabetic rodents. The intestinal transit of charcoal meal can be assessed by the modified Janseen technique.

4.1.3 Structural Biomarkers of Diabetic Neuropathy in Rodents:

A. Decrease in Epidermal Nerve Fibre Density:

Quantification of epidermal nerve fibre density by skin biopsy is an evolving method to diagnose and stage peripheral nerve disorders. Risk factors corresponding to diabetes have shown that IENF density is a good indication of diabetic neuropathy, study of etiologic mechanisms, and evaluation of therapeutic success.

B. Large Variation in Axonal Diameter:

Through longitudinal visualisation of muscle spindle afferents, it's evident how varied axonal width and IRD [i.e., interrotational distance] are. A study in STZ-induced C57BL/6 diabetic mice suggests that muscle spindle la fibres have a high variability in their axonal width/diameter and IRD as a group and within individual muscle spindles. By utilising this technique or method for detecting the effectiveness of therapies designed to improve large-fibre DN, we have developed a new means to investigate the therapeutic approaches for this disorder.

C. Reduction of Axonal Number and Caliber:

This indicates the morphological effects of diabetic pathology on large-diameter axons [fibre], which has been demonstrated in studies performed on diabetic mice. In one study, diabetic mice had significantly smaller mean fibre areas and thinner myelin sheaths on tibial fascicles compared to control mice, but the density and the total number of fibres were not affected. In contrast, there was no associated change in the diameter of the sural nerves, which have a predominant number of sensory axons and a few axons that are autonomic in nature.

4.1.4 Molecular Biomarkers:

A. Down-Regulation of Structural Protein [Neurofilament, Tubulins, Nerve Growth Factor]:

In addition to their role in development and regeneration, NGF and structural proteins are important in maintaining sensory and autonomic neurons as well as the axons of these neurons and will likely to be used as biomarkers for neuropathy in an animal model of diabetes. There have been multiple indications that levels [both in serum and nerves] of these materials are decreased in a diabetic state. Additionally, the diminished capacity of nerves to regenerate in diabetes may be the result of an inappropriate neurotrophic tone; this may reflect the lessened synthesis, release or response to neurotrophins, such as NGF and structural proteins, in the axons of sensory and autonomic nerves.

B. Down-Regulation of Neurotrophin Receptors:

Neurotrophins bind to two classes of receptors: the tyrosine receptor kinases [Trks—TrkA, TrkB, and TrkC] and the low-affinity receptors, "p75".

C. Biomarkers of Oxidative Stress:

Signs of oxidative stress were present in the brains of rats that had developed long-term diabetes following IP administration of STZ. Malondialdehyde [MDA] and nitric oxide [NO] levels were significantly elevated, while the antioxidant system of glutathione [GSH], glutathione reductase [GR], glutathione peroxidase [GPX], glutathione S-transferase [GST], and superoxide dismutase [SOD] showed significantly reduced activity compared to controls.

Other biomarkers for oxidative stress reported in diabetic rodent species at the molecular level include up-regulated markers for neurotoxicity [i.e., nitrotyrosine], elevated levels of intracellular calcium, and over-activation of poly [ADP-ribose] polymerase. Additional biomarkers, including activated caspases, the receptors for advanced glycation end products [RAGE], and the nuclear factor kappa B [NFkB], all contributed to polyol accumulation in diabetic rodents through increased levels of nerve sorbitol or fructose.[30]

5. LIMITATIONS & TRANSLATIONS:

A significant impediment to the successful translation of a treatment from preclinical to clinical drug testing is related to how adequate an animal model of the disease has been

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selected [preclinically] and how adequately the outcome measures have been selected. Many animal models that are proposed to mimic the painful neuropathy associated with human conditions include: high-dose STZ in mice and rats [many of which are genetically altered], and diets of a type that are designed to induce T1D and T2D in experimental animals. No rodent model, however, is an accurate mimic of human neuropathy syndrome. Another major limitation to accurately measuring ongoing pain-associated behaviour is the difference in how pain is measured in humans vs. rodents; namely, most neuropathic pain patients have an ongoing level of pain independent of any noxious stimuli [i.e., touch], which is especially difficult to assess in the rodent models. To facilitate the predictive nature of future preclinical studies to better estimate clinical circumstances, various design considerations should be expanded, such as: multiple endpoints measured, including both evoked pain and ongoing pain; assessing comorbidity; assessing quality of life; and including genetic variability in the animal model used [e.g., outbred strains]; as well as both male and female animals. Another very important consideration to improve clinical translation of preclinical studies is the design of the clinical trials themselves [i.e., how one assesses pain intensity as a primary endpoint vs. a combination of pain intensity, disturbance, and loss of function—pain severity].[27]

Preclinical studies have significant limitations associated with the many chemically induced diabetic models that have been used for diabetes research and the insulin depletion that consequently biases the study of T1D. While most of the time drugs of injections of insulin are used to maintain blood glucose levels within the normal range in humans, typically these drugs and injections cannot be used in animals. Which lead to a much more aggressive phenotype being observed in the animals than would otherwise be the case in humans. These contraindications, coupled with the overall lack of agreement on the protocol for the induction of diabetes in animals, along with differences in dose, age of animal and duration of the observation period, will lead to significant differences in the protocol and the experimental endpoints used. Another major limitation of both preclinical and clinical studies is related to the assessment of pain in both animals and humans; most estimates indicate that approximately 30% of clinical and preclinical studies evaluate the pain threshold or other symptoms of pain in both animals and humans but are not comparable using the various pain assessment methods available. To facilitate a clearer understanding of both the preclinical and clinical levels of diabetes and enable better translational and reverse translation approaches, it will be necessary to develop comparable measurements and assessments used to evaluate diabetes in studies.[43]

6. THERAPEUTIC APPROACHES:

Treatment of NP uses a tiered approach to therapy. The goal of treatment of NP is to reduce the amount of suffering experienced by the patients and to improve functional outcomes by reducing the intensity of pain and improving overall quality of life. Many first-line treatments [e.g., opioids, NSAIDs] have been used successfully for many individuals, but second-line treatments also provide a different class of medications for individuals who require something other than the first-line therapy options. Topical medications represent an important group of second-line agents that may utilized the treat NP; however, they are typically reserved for patients who did not find adequate pain relief after trialling first-line or second-line agents, or experienced side effects that made the initial agents intolerable. In general, topical agents exhibit less extensive clinical data to support their use than that of first-line agents or many second-line agents; they also exhibit approval for fewer indications and have a high incidence of adverse effects. However, topical agents target specific mechanisms involved in the neurobiology of pain and may be utilised successfully in patient populations.[44]

The Special Interest Group, in NP, recommends gabapentinoids [pregabalin, gabapentin], tricyclic antidepressants [TCAs], and selective serotonin-norepinephrine reuptake inhibitors [SNRIs] as the first-line therapy option for the treatment of NP using peripheral nerves; Lidocaine, capsaicin, and tramadol are recommended second-line therapy options. Strong opioids such as morphine or oxycodone and botulinum toxin-A [BTX-A] are considered third-line therapy options in the management of peripheral nerve neuropathic pain.[45]

7. CONCLUSION AND FUTURE DIRECTIONS:

This article presents a review of the rodent models currently being used to study neuropathic pain, with a focus on models for diabetic peripheral neuropathy, as well as providing general overviews of other types of models used to study the effects of neuropathy. Overall, it has been determined that animal models have helped us understand the processes that cause neuropathic pain and can serve as useful indicators of potential treatment efficacy by linking behavioural changes resulting from pain to both conduction and structural damage to nerves. Existing treatment options for neuropathic pain have not resulted in a definitive resolution of the cause of pain. It is anticipated that with a combination of innovations and improvements in the field and through a more streamlined protocol for conducting clinical trials with a greater appreciation for the principles of neuroplasticity, there will be improved treatment strategies to manage NP and ultimately, there will be successful transfer of the scientific knowledge gained through this research to patients. By improving the knowledge gained from preclinical studies to clinical applications through creating a consistent endpoint for comparing animal models to humans and quality of life metrics, we can improve the patient's overall quality of life.

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