ANIMAL MODELS FOR ASSESSMENT OF NEUROPATHIC PAIN

Abstract

This chapter focuses on the Animal models used in assessment of neuropathic pain conditions. It contains details on the several forms of neuropathic pain, such as peripheral and central neuropathy, and how their mechanistic involvement of different pathways may lead to the development of neuropathic pain. The primary emphasis is on the use of animal models.

Keywords: Animal models, Neuropathicpain, Nerve injury models

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I. NEUROPATHIC PAIN

Neuropathic pain (NP) is frequently defined as a persistent scorching or shooting pain broughton by nerve breakdown or damage in the somatosensory nervous system, which affectects peripheral nerve fibers such as Aβ, Aδ and C fibers and central neurons. According to, NP is defined by the International Association for the Study of Pain (IASP) as ”pain caused by damage or disease affecting the somatosensory nervous system.” This occurs due to certain conditions such as metabolic disorders, infection, cancer,trauma, medicines, and toxins.

NP is marked by spontaneous greater pain reaction to stimuli that are painful or innocuous. The somatosensory nervous system is involved with theconscious awareness of sensations that come from the muscles, joints, skin, and fascia, such as touch, pressure, pain, warmth, position, movement, and vibration. Amputation, alcoholism, chemotherapy, diabetes, facial nerve problems, HIVinfection, multiple myeloma, multiple sclerosis, arthritis in the spine, spine surgery, syphilis, thyroid issues, vitamin B12 deficiency, Charcot-Marie-tooth, post-herpetic neuralgia, post- sternotomy, post-mastectomy, post-thoracotomy, and post-herniorrhaphy are some common causes of NP. Lesions in the somatosensory nervous system brings undesirable changein the transmission of sensory signal to an electric signalin the nervous system. NP shows gloves and stockings pattern of distribution, it mainly affects feet, calves, hands and forearms. NP brings changes or modulation, or alteration in pain signaling, pain transmission neurons, inhibitory interneurons and descending modulatory control systems, Ion channel, second-order nociceptive neurons and pain mechanisms.

The NP broadly divides in two types central neuropathy and peripheral neuropathy

1. **Central Neuropathy:** According to the IASP, central pain is pain that originatesfrom or is brought on by a primary CNS injury or dysfunction. A group of persistent NP disorders known as central pain syndrome are brought on by CNS injury.Central pain may arise after a traumatic brain injury and spinal cord injury, like syringomyelia, multiple sclerosis, stroke (infarction or hemorrhage), Parkinson’s disease, tumors, and epilepsy. The characteristics of central pain are it is chronic, disabling, and resistant to treatment due to which it has a major influence on mood andlifestyle quality of the patients’ sufferering from it. The clear diagnostic criteria for central pain are not established, whichmakes the diagnosis difficult. In recent times the NP scale (NPS) is the only validated tool for assessment of central neuropathic pain. The available treatments are only effective in reducing pain to some extent. Approximately 8% of stroke patients and 25% of multiple sclerosis individuals experience central pain and 50% of spinal cord injury patients. Example of Central NP includes harm to thespine or brain, a stroke, or multiplesclerosis.

2. **Peripheral Neuropathy:** is a condition developed due to damage to the peripheral nerves. Peripheral nerves are located outside the brain and carry signals to and from the brain andspinal cord. It is the common, chronic, disabling sometimes mortal condition that causes sufferings to the patient. Peripheral neuropathy has heterogenicity in etiopathogenesis, manifold pathology, and diversified severity. Glove and stocking sensoryloss, absence of tendon reflexes, distal wasting and weakness, and progressive polyneuropathy are the

hallmarks of this condition. Chronic NP can develop as a result of peripheral nerve injury in several ways. It is evident that hyperglycemia is crucial for the onset and development of diabetic neuropathy and other microvascular consequences of diabetes. Which are Peripheral nerve damage, uncomfortable polyneuopathies, or Rdaiculopathies are example of peripheral mainly driven by Trigeminal or postherapeutic neuralgia, neuropathy.

II. PATHWAYS INVOLVED IN NEUROPATHIC PAIN

1. **Diabetic Neuropathy and the Polyol Pathway:** Extremely high intracellular glucose levels result from hyperglycemia in nerve cells, which also results in the glycolytic pathway being saturated. The enzymes aldose reductase and sorbitol dehydrogenase convert extra glucose into sorbitol and fructose.

   Myoinositol is decreased as sorbitol and fructose buildup, which in turn affects the activity of themembrane Na+/K+ATPase, impairs axonal transport, and damages the structural integrity of neurons. According to Brownlee 05, the aldose reductase (AR)-mediated conversion of glucose to sorbitol depletes the antioxidant nicotinamide-adenine dinucleotide phosphate(NADPH), which is necessary for the renewal of reduced glutathione (GSH). Nitric oxide synthase requires NADPH asa cofactor; when NADPH levels are low, nitric oxide synthase produces less nitric oxide,

   Which results in less vasodilation, which lowers blood flow to the nerve. Galactosaemic animals' peripheral nerve ATPase causes myoinositol levels to drop. It triggers a chain of events that includes decreased membrane Na+/K+ATPase activity, intra-axonal sodium accumulation, and structural breakdown of the neuron as a result.

2. **Protein Kinase-C (PKC Activity in Diabetic Neuropathy:** PKC pathway is another way by which hyperglycemia damages the tissue. Diacylglycerol (DAG) concentration is stimulated by high glucose levels, and this triggers the PKC pathway. Increased PKC-ß-isoform production has been linked to the vascular endothelial growth factor (VEGF), PAI-1, NF-B,and TGF-ß are angiogenic proteins that are overexpressed alongwith diabetic complications.

3. **Hexosamine Pathway in Diabetic Neuropathy:** Considered an important mediator in the pathophysiology ofdiabetes induced oxidative stress and its consequences. Fructose -6 phosphate is a metabolic intermediate step in glycolsis. Some fructose -6-phosphate is diverted from the glycolytic pathway to the hexosamine pathway during the breakdown ofglucose. The hexosamine pathway experiences increased flux under hyperglycemic circumstances, which leads to an excess of GlcNAc and abnormal gene expression changes.

4. **Advanced Glycation End Products (Age) In Diabetic Neuropathy:** AGE, which is produced as a consequence of non-enzymatic glycation of proteins, nucleotides, and lipids in hyperglycemia,may interfere with integrity and mechanisms for neuronal repair.

Table 1: Non-Pharmacological remedies for the Prevention of Peripheral Neuropathy

Non-Pharmacological Remedies	Examples
Hypnosis	Altered state of consciousness
Relaxation	Deep breathing and stretching
Comfort therapy	Exercise, applying heat or cold, massage therapy, theatre therapy, music therapy strengthening and Desensitization
Neurostimulation	Acupuncture and TENS (transcutaneous electrical nerve stimulation)
Others	Acupuncture and TENS (transcutaneous electrical nerve stimulation)

III. ANIMAL MODELS FOR ASSESSMENT OF NEUROPATHIC PAIN

Animal models play a crucial role in studying neuropathic pain, as they provide valuable insights into the underlying mechanisms and aid in evaluating potential therapies. NP is acomplex state that arises from dysfunction of the neurological system, resulting in persistent pain signals and abnormal sensory processing. By utilizing animal models, researchers caninvestigate various aspects of neuropathic pain, including itsetiology, pathophysiology, and treatment options. One of the primary advantages of animal models is their ability to replicatecertain features of human neuropathic pain. These models are designed to mimic specific neuropathic conditions by inducing nerve injury or disease-like symptoms, allowing researchers to study the associated pain behaviors and physiological changes.

By observing animals' responses to pain stimuli and analyzing their neurobiological alterations, scientists can gain insights into the mechanisms involved in neuropathic pain. Animal models also enable researchers to explore the effects of potentialtherapies for neuropathic pain. They provide a controlled experimental setting where interventions can be tested and their effcacy assessed. This includes pharmacological interventions, such as administering analgesic drugs or investigating novel compounds, as well as non-pharmacological approaches like physical therapy or neuromodulation techniques. Animal models allow for the evaluation of treatment outcomes, dose-response relationships, and potential adverse effects, providing valuable information to guide clinical studies in humans. Thereare various animal studies available for the screening of NP and research has shown the effectiveness of each model and theexcellent effects of natural products on neuropathic pain.

The various screening models are listed in Figure 1. Here are some typical animal models for NP research includes:

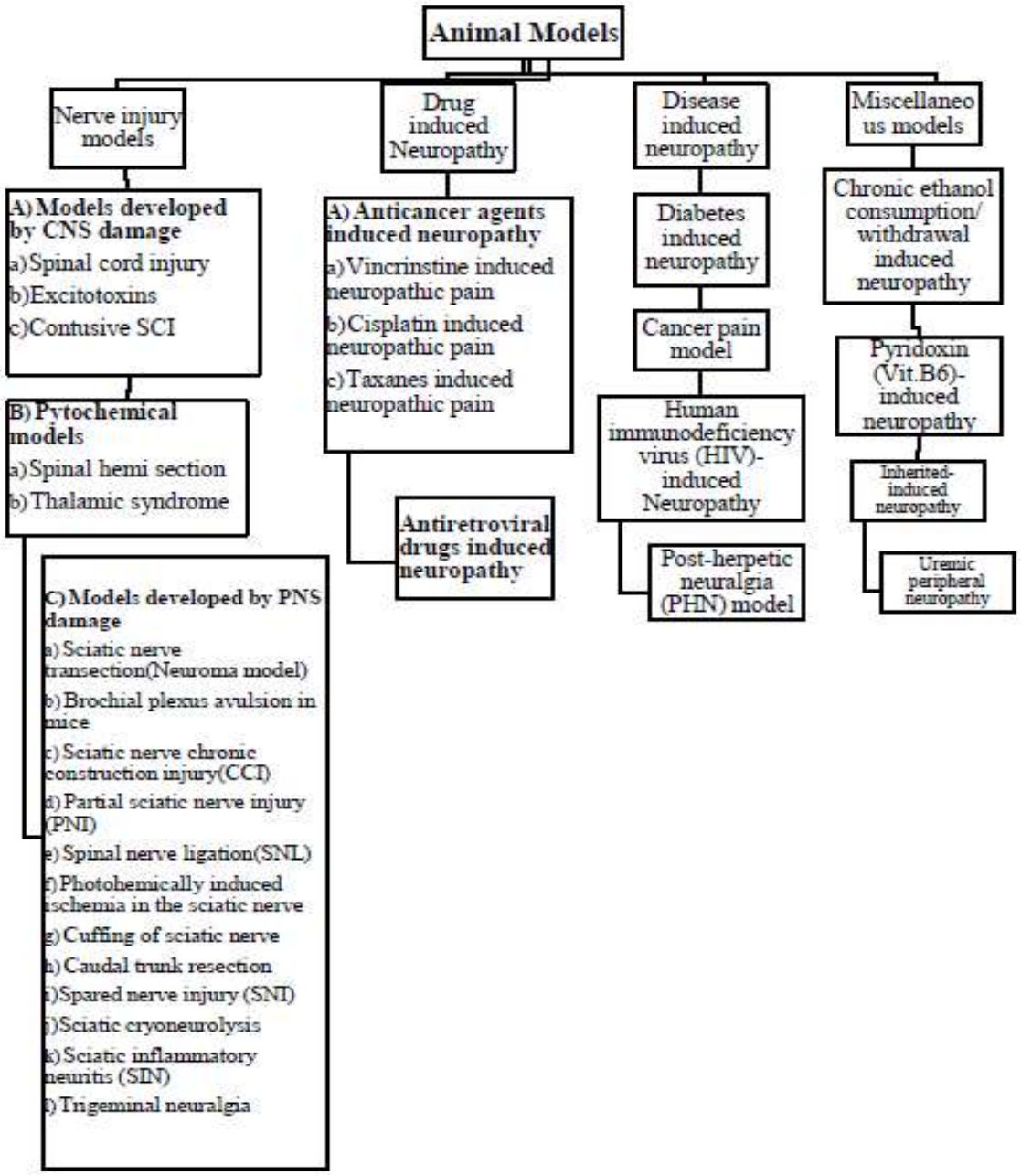

Figure 1: Classification of Animal Models

IV. NERVE INJURY MODELS

1. Model Developed by CNS Damage

- **Spinal Cord Injury (SCI) Models:** SCI models involve contusion, compression, or transection of the spinal cord. Thesemodels not only mimic the sensory and motor deficits

observedin human spinal cord injury but also lead to the evolution of NP symptoms.NP model by SCI is developed by using one the following technique-Contusion or weight dropping

- Spinal cord compression
- Excitatory neurotoxins
- Photochemical-induced ischemia
- Spinal cord transaction
- Crushing of the spinal cord
- Clip Compression Injury
- Spinal Cord Displacement
- Canal Stenosis

- **Spinothalamic Tract Lesion:** Spinal cord injury by any of the above techniques damages the nerves that transport the motor and sensory signals from and to spinal cord and brain which leads to the NP characterized by hyperalgesia and .Various mechanisms of SCI are studied. The mechanism of spinal cord injury includes Peripheral mechanism, Spinal mechanism and Supraspinal mechanism.

- **Model Developed by PNS Damage:** Spinal Nerve Ligation (SNL) Model: In this model, a specific spinal nerve is surgically ligated, leading to the evolution of NP symptoms in the corresponding dermatomes. It mimics certain aspects of nerve damage-related NP observed in humans.

  The SNL framework serves as a technique for researching medication for neuropathic pain that is chronic. The experimental drugs with analgesic qualities that are utilized as remedies for persistent neuropathic pain are found using this model. In order to produce peripheral pain, the L5 and L6 spinal nerves are surgically ligated.

- **Chronic Constriction Injury Model (CCI):** This model was developed in rats by Bennett and Xie in 1988 & in mice by Sommer in 1997, model was designed in such a way that it mimics the peripheral nerve damage in patients of NP. This model is produced under anesthesia by the constriction of the nerve most commonly sciatic nerve and in some cases infraorbital nerve and the median nerve, in which the nerve is tied using several ligatures resulting in incomplete nerve injury involves epineural inflammation, intraneural edema, and Wallerian degeneration. Pain hypersensitivity testing is done by measuring the mechanical and thermal withdrawal threshold & latency. This model involves the placement of a ligature around a peripheral nerve,resulting in sustained compression and chronic constriction.

  It induces neuropathic pain-like behaviors and is particularly useful for studying peripheral nerveinjury-induced pain.

- **Spared Nerve Injury (SNI) Model:** In this model, some of the major branches of a peripheral nerve are carefully spared, while others are injured. It produces robust and long-lasting behavioral changes, allowing researchers to study mechanisms of both allodynia (pain from non-painful stimuli) and hyperalgesia(increased sensitivity to painful stimuli).

- **Partial Sciatic Nerve Injury Model (PNI):** In this model the peripheral neuropathy is developed by tight ligation of peroneal nerve or tibial nerve. Unlike STZ- induced diabetic animals, PNI induced neuropathic animals were notchronically ill, growth rate is not reduced, polyuria is not observed, diarrhea, and enlarged and distended bladders is not found. Signs and symptoms of neuropathy develop after 1 week of surgery. This model initiates long-lasting mechanical hyperalgesia but thermal hyperalgesia is not produced by this model. PNI is evaluated by, Morphine and L-Baclofen. The major limitationof PNI is that the major pathogenesis was not characterized.

V. DISEASE INDUCED NEUROPATHY

1. **Diabetic Neuropathy Models:** Animals with experimentally induced diabetes, such as streptozotocin (STZ)-treated rodents, can develop peripheral neuropathy resembling diabetic neuropathy observed in humans. These models are utilized tostudy the underlying diabetic NP.

- **Streptozotocin-Induced Diabetic Neuropathy in Rats and Mice:** The classic model was developed by Jakobsen and Lundbeckand the classic model was developed by Filho and Fazan forphrenic nerve neuropathy in rats. The toxicity produced by Streptozotocin is due to presence of nitrosoamide moiety, it damages the DNA of insulin secreting beta cells present in pancreas and produces toxicity. The level of damage to beta-cells is dose dependent. Due to the similarity with glucose STZ get easily transported through glucose transporter GLUT2.The diabetes is developed in animals bygiving a single injection of STZ through an intraperitoneal or intravenous route. Different factors such as age, strain, and species are responsible for the sensitivity of animals to STZ.The development of Diabetic neuropathy using STZ reducesdiameters of the myelin sheath, axon, and nerve fiber, showsimpairment in motor performance and significantly decreases the myelination of the phrenic nerves and the right and left fascicular regions. As STZ increases AR activity,oxidative-nitrosative stress, toll-like receptor 4, protein kinase C, PARP and ACE activations, C-peptide deficiency, impaired neurotropism and proinflammatory response streptozotocin induced diabetic animal models are extensively used tounderstand diabetic NP.

- **Alloxan Induced Diabetic Neuropathy:** Alloxan is very unstable weak barbituric acid derivative which is first isolated by Brugnatelli (1818) Alloxan is selectively up taken by the beta cells of pancreas due to the similarity with glucose in molecular shape and hydrophilicity, it gets accumulated in the cells andproduces diabetogenicity. Alloxan produces diabetes through a partial degeneracy of the beta (β) cells present in islets of pancreas and brings a considerable change in insulin production by β-cells both

qualitatively andquantitatively. It is First time usedas McLetchie. It produces type 1-DM by 24-48 hrs of administration of single dose by intraperitoneal route.

VI. DRUG INDUCED NEUROPATHY

1. **Anticancer Agents Induced Neuropathy:** Chemotherapy have many side e effect. s, from which peripheral neuropathy is the usual side effect. Chemotherapy damages somatosensory nervous system which may be the reason for development of peripheral neuropathy. The antineoplastic agents used in chemotherapy damages the healthy cells including nerves that affect feeling and movement in the hands and feet. In chemotherapy the chances of development of peripheral neuropathy is 80- 90 %.Various Antineoplastic agents are used by researchers to develop the CIPN model such as paclitaxel, cisplatin, carboplatin, and oxaliplatin and others such as vincristine, thalidomide, suramin, and bortezomib.

- **Vincristine-Induced Neuropathic Pain:** Vincristine is used as antineoplastic agent. It belongs to the vinca alkaloid family. It is used in treatment of malignant tumors, lymphoma and leukemia. Use of vincristine causes peripheral neuropathy which limits its use. Vincristine develops neuropathy by altering microtubular structures of intracellular tubulin and damages peripheral axons results in dysfunction in primary afferent fibers like Aβ-, Aδ-, and C-caliber, which results in dose-dependent neuropathy. The early signs of neuropathy by vincristine administration is paranesthesia, which progresses to hyperesthesia.

  This model developed by giving IV injection or by continuous intravenous infusion of vincristine. The suffcient dose for development of neuropathy by vincristine is as low as 50 µg/kg. It induced consistent and long- lasting signs and symptoms of neuropathy like Allodynia, hyperalgesia(mechanical) and hypoalgesia (thermal) similar to the vincristine treated cancer patients, which makes it a potential study tool for studying the pharmacological mechanisms of vincristine induced NP.

- **Paclitaxel-Induced Neuropathic Pain**: Paclitaxel, a vinca alkaloid is a potential antineoplastic agent as a treatment for breast cancer, head and neck cancer, melanoma and ovarian cancer. By inhibiting the polymerization of microtubules and binding to tubulin, paclitaxel causes sensory neuropathy and myelosuppression and interferes with mitosis.

  In models receiving low doses of paclitaxel, loss of pain perception, morphological abnormalities, neurophysiologic problems, and changes to motor function are rare. So it is better to study these changes with the model of higher doses. The Paclitaxel-Induced NP model proved that, they produced slightest effects on the rats health and mimics the conditions developed in patients treated with taxens, which makes it a potential study tool for studying the pharmacological mechanisms.

- **Oxaliplatin-Induced Neuropathic Pain:** It is a third-generation antineoplastic drug based on platinum that is used to treat colorectal cancer that has progressed. Oxaliplatin

develops neuropathy by inhibiting DNA synthesis and the replication of DNA, damages the neuronal cell bodies, decreases SNCV and axons in peripheralnerves are deteriorating. Development of neuropathy at combined dosages (36 and 48 mg/kg i.p.).

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