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Review Article

NEUROPATHIC PAIN: POSSIBLE STRATEGIES FOR PATHOPHYSIOLOGY AND MANAGEMENT

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Abstract
Pain is an unpleasant sensation that has both physical and emotional components. The physical part of pain results from nerve stimulation. Pain is mediated by specific nerve fibers that carry the pain impulses to the brain where their conscious appreciation may be modified by many factors. *Neuropathic pain* results from damage to or dysfunction of the peripheral or central nervous system, rather than stimulation of pain receptors. Chronic pain results from injury to the nervous system. The injury can be to the central nervous system or the peripheral nervous system. There are various factors (chemical excitation of nonnociceptors, recruitment of nerve outside of site of injury, excitotonicity, sodium channel, ectopic discharge, central sensitization and sympathic over stimulation) involve in the pathophysiology of neuropathic pain. In recent years, it is increasingly recognized that non-neuronal cells such as immune cells (macrophages, lymphocytes) and glial cells in the PNS (schwann cells and satellite cells) and in CNS (astrocytes and microglia) also play a critical role in chronic pain processing. Symptoms of neuropathic pain are mainly associated with numbness, burning, tingling, dysesthesia, hypergesia, allodynia. The concomitant symptoms are includes disturbance of sleep, mood, sex life and recreation. One of the more invasive but effective treatments for chronic neuropathic pain is neurostimulation. The treatment is based on creating paresthesias due to electrical stimulation in the affected and painful area

Keywords: - : Neuropathic pain; inflammation

Introduction
Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. The pain may be nociceptive, neuropathic, neuroplastic and inflammatory. Neuropathic pain is one of the chronic painful conditions. Neuropathic pain represents pathological change in both peripheral and central nervous system and may results nerve damage, nerve damage may be caused by a variety of insults including traumatic nerve injury, metabolic diseases, viral infections, or stroke etc. (1 Combination of positive and negative sensory symptoms often occurs in patients of neuropathic pain. These can be paresthesias (numbness or tingling), dysesthesias (electric shock phenomenon), hyperesthesia (increased sensitivity to mild painful stimuli), hyperalgesia (increased sensitivity to normally painful stimuli), hyperpathia (pain produced by sub threshold stimuli), spontaneous pain and allodynia (pain produced by normally non-painful of stimuli) (2).

The pathophysiology of NP is still not properly understood. Several hypotheses have been suggested like oxidative stress, nitric oxide pathway and neuro-inflammatory cascade may involve in pathogenesis of NP. However, it is now clear that spinal cord glial cells (microglia and astroglia) importantly contribute to pain facilitation (3). Upon activation following nerve trauma and/or inflammation, glia cells release proinflammatory cytokines and other mediators, which facilitate pain transmission. Both astroglial and microglia activation occur in the spinal cord in models of neuropathic pain . Oxidative stress plays a key role in peripheral and central sensitization at peripheral sites and spinal cord (4). Oxidative stress imposes on the cells as a result of (i) due to increase in free radical generation (ii) weak antioxidant defense and (iii) a failure to repair oxidative damage (5). The main source of ROS in vivo is aerobic respiration, although ROS is also produced by paroxysmal- b oxidation of fatty acids, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine

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metabolism and tissue specific enzymes. Unchecked, ROS may attacks many key molecules including enzymes, membranes, lipids and DNA. Under normal conditions, excessive ROS neutralized and cleared from cells by the action of superoxide dismutase (SOD), catalase or glutathione, as well as antioxidant vitamin C and E (6). In pathological conditions, intracellular ROS level is raised due to its increased production which further cause cell damage ranging from cytoplasmic swelling to death (7). Obviously, removal of excessive ROS is often important in restoring normal conditions. In neurons, glutathione system as free radical scavenger.

NO, a free radical synthesized from L-arginine by calcium-dependent constitutive NO synthase *(NOS)* isoforms, including neuronal NOS *(nNOS)* and endothelial *(eNOS),* orthe calcium-independent inducible NOS *(iNOS)* that requires activation by endotoxin or cytokines (8). NO produces an increase in intracellular cyclic guanosine 3, 5′-monophaosphate (cGMP) through activation of soluble guanylatecyclase. Evidence has shown the involvement of both central and peripheral involvement of NO in nociceptive processing. The release of NO is apparently requires for the maintenance of hyper excitability, as high doses of NO-donors cause hyperalgesia (1) while, low doses reduce hyperalgesia suggesting a dual role of NO. It is now well reported that NO plays an important role in neural transmissions and act as a second messenger in the central and peripheral nervous system. There is considerable evidence which indicates that NO cGMP pathway is involved in central nociceptive processing at both spinal and supraspinal levels. Intrathecal administration of NO donors such as 3-morpholinosydnonimine (SIN-1), *S*- nitroso*N*- acetyl D-penicillamine(SNAP) and L-arginine induced hyperalgesia. However, Sousa and Prado, (2001) showed that intrathecal administration of low dose of sildenafil produced antinociception while in high dose increased the mechanical allodynia induced by chronic sciatic nerve ligation in rats. The possible explanations for the nociceptive processing of NO-cGMP pathway include different doses via the drug, drug specificity, route of administration, distribution and pharmacokinetics. More importantly, NO acts as an antioxidant in certain environments and prevents lipid peroxidation (10). However, when O_2 increases, NO reacts with the O_2 to form peroxynitrite and becomes a prooxidant. This prooxidant activates many inflammatory markers which cause neuronal damage. It is now believed that mechanism responsible for hyperalgesia in chronic pain may involve not only NO itself, but also the product of its reaction with superoxide radicals, the peroxynitrite (7).The functional role of NO-cGMP pathway in the spinal mechanism of chronic neuropathic pain remains to be clarified.

The treatment of the underlying causative condition is central to the management of neuropathic pain, the non-pharmacological measure include psychological technique, acupuncture, transcutaneous electrical nerve stimulation and the pharmacological option include classes of agents with efficacy demonstrated in multiple, randomized, controlled trials for neuropathic pain like topical analgesics, anticonvulsants, antidepressants, opioids etc. Curcumin, commonly called diferuloyl methane, is a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa*. Low concentrations of curcumin have also been found to inhibit nitric oxide production, as measured by the amount of nitrite released into the culture medium (11). Results indicate that curcumin act as a free radical scavenger, inhibits TNF-α production and development of peripheral neuropathy in streptozotocin-induced diabetic rats (12). Curcumin is also known to reduce amount of peroxynitrite formed by the reaction between oxygen and nitric oxide, generated from sodium nitroprusside (13). Studies have shown that curcumin is both a nitric oxide scavenger and an inhibitor of inducible nitric oxide synthase (iNOS) expression, low levels of which cause correlate with anti-apoptotic function and poor survival.*Withaniasomnifera*(Dunal), popularly known as Indian Ginseng, belongs to the familySolanaceae, commonly known as Ashwagandha, a subtropical undershrub, (14).reported that glycowithanolides (the active principles of WS) alter the cortical and striatal antioxidant enzyme activities (superoxide dismutase, catalase and Glutathione peroxidase) in rats. A 30-day treatment with *Withaniasomnifera* root produced a significant decrease in lipid peroxidation, and an increase in both superoxide dismutase and catalase**,** indicating that WS root powder cause free radical scavenging activity (15). A number of earlier investigations have indicated that WS exhibits an activity profile, which may reflect putative anti-oxidative stress effects. Thus WS, or its major active principles, have anti-inflammatory and they inhibit the nitric oxide synthesis. Local injection of ketorolac, a nonselective COX inhibitor, into the ipsilateral plantar side or into the injured region of the sciatic nerve reversed the mechanical allodynia induced by PSNL for more than 5 days this suggest the role of COX in neuropathic pain. Not only COX, there many other pathways (form the arachidonic pathway i.e. LOX one.) which play an impotent role in neuropathic pain of all the

initial steps in leukotriene biosynthesis (16).

Current treatments those are available for neuropathic pain shows only symptomatic effect. Some new neuroprotective strategies needed for the treatment of neuropathic pain. Present study is an attempt to explore neuroprotective strategies for the treatment of neuropathic pain. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone mainly produced by the pineal gland in all vertebrates and follows a circadian pattern. Several groups have shown that melatonin reverses chronic and acute inflammation. Melatonin treatment also causes an important reduction of nitric oxide (NO) and malondialdehyde (MDA) levels, these two compounds that are closely related to oxidative stress. Likewise, melatonin inhibits the inflammatory response and inducible NO synthase (iNOS) isoform expression in the pleurisy model. In the brain, minocycline has been shown to produce neuroprotective effect by inhibiting inflammation, decreased free radical formation by inhibiting inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and inhibits the caspase-1 in experimental model of Parkinson's and Huntington's diseases, and prevented *N*-methyl-D-aspartate mediated neurotoxicity (17). Recently, its antihyperalgesic and antiallodynic effects have been demonstrated in models of arthritis, spinal nerve transection, sciatic inflammatory neuritis (18). In mice with partial tight ligation of the sciatic nerve, melatonin significantly reduced thermal hyperalgesia and allodynia effect, which was partially reversed by L-arginine, suggesting that NO synthase are involved in melatonin antihyperalgesic and antiallodynic effect.

Therefore, present study has been designed to investigate the possible role of neuroprotectants and to determine the role of microglia cell and arachidonic pathway in experimental model of neuropathic pain. Attempt has also been made to explore some novel combinational strategies for the treatment of neuropathic pain.

NEUROPATHIC PAIN:

Neuropathic pain is a consequence of peripheral nerve injury applied to a syndrome of varying pathologies. It may occur in any part of the neuron and at any point from peripheral terminals to central terminals. The International Association for the Study of Pain (IASP) defines neuropathic pain states as disorders that are characterized by lesions or dysfunction of the neural system(s) that under normal conditions transmit noxious information to the central nervous system(2).

The abnormal nature of neuropathic pain means that pain is often disappear from any area of tissue damage, and degree of pain often does not correlate with the apparent extent of peripheral tissue damage. While some patients may suffer neurological deficits indicative of a significant nerve injury but remain free from pain, others report severe pain despite retaining normal nerve function. Clinically, patients often describe ''burning'' pain and intermittent ''shooting'' or ''electric shock'' sensations. Neuropathic pain may be evoked in response to stimuli such as touch, temperature change, or movement, or may be spontaneously occurring. Evoked pain as a result of nerve damage takes two forms (as defined by the IASP): **Allodynia**-Pain due to a stimulus that does not normally provoke pain. Allodynia may be provoked by innocuous touch stimulation (mechanical allodynia) or cooling (cold allodynia). **Hyperalgesia**- An increased response to a stimulus that is normally painful. Hyperalgesia in individuals with neuropathic pain is typically evoked by heat stimulation. In addition to pain, spontaneous parasthesias and dysesthesias—abnormal sensations such as numbness, itch, and tingling, which are unpleasant but not painful—are commonly present (19). Neuropathic pain occurs as a consequence of a variety of injuries, trauma, infection, disease, metabolic aberrations, nerve compression, inflammation, and chemotherapy. Often regarded as a symptom of other pathologies, a warning of an underlying disease state, pain has often been undertreated due to an emphasis on treating the perceived pain-causing disorder. Indeed, neuropathic pain is the consequence of multiple mechanistic changes occurs within peripheral and central nervous system, rather than etiological consequence of a single event and thus, is best viewed as a group of disorders of the nervous system. A patient suffering from a chronic neuropathic pain is likely to suffer from more than one modality of pain, each with a different impact on daily life. Ongoing pain will often lead to disturbed sleep, while any associated sensory allodynia will affect normal behavior, even to the extent of impairing the ability to wear clothing. Pain is often compounded by dysesthesia and impaired motor function. From the diversity of causes, it follows that a particular neuropathic pain syndrome will affect a heterogeneous population in terms of their symptoms and underlying mechanisms. In turn, their treatment and prognosis will inevitably differ. The impact of neuropathic pain is evident from the molecular level to cognitive and social aspects. It is not just a source of physical discomfort, but engenders a wide range of CNS disturbances, including sleep dysfunction, depression, and anxiety (20). The degree to which these changes are manifested are controlled by environments and situations in which they occur, and therefore physiological, genetic, psychological, and social

factors bear upon the incidence and perception of pain. Neuropathic pain has the potential to become a greater problem in the future without adequate therapies. With an aging population, the incidence of many neuropathic pain syndromes, several associated with aging, will inevitably increase. The need for a greater understanding of the mechanisms underlying neuropathies and associated pain is, therefore, greater than ever if viable and effective treatments are to be developed.

Classification of Neuropathic Pain (NP)

The type of damage or related pathophysiology causing a painful neuropathic disorder can be classified as the following (22)

a) **Mechanical nerve injury**, e.g. carpal tunnel syndrome, vertebral disk herniation;

b) **Metabolic disease**, e.g. diabetic polyneuropathy;

c) **Neurotropic viral disease**, e.g. herpes zoster, human immunodeficient virus (HIV) disease;

d)**Neurotoxicity,** e.g. by chemotherapy to treat cancer or tuberculosis;

e)**Inflammatory and/ or immunologic mechanisms**, e.g. multiple sclerosis;

f)**Nervous system focal ischemia**, e.g. thalamic syndrome (anesthesia dolorosa);

g)**Multiple neuro transmitter system dysfunctions**, e.g. complex regional pain syndrome (CRPS).

Classification of neuropathic pain is an important but still an unsettled issue. A large range of etiologies indicate that the prevalence of neuropathic pain may be high in the general population. However, epidemiological studies did not allow estimation of the overall prevalence of neuropathic pain in the general population, but crude 1-3% estimation in range has been reported (23). In theory neuropathic pain is easy to distinguish from other conditions but in practice, they are both difficult to identify and treat.

Clinical Findings in Neuropathic Pain

Patient with neuropathic pain presents with a combination of following pain type:-

(a) Spontaneous constant pain which fluctuates in intensity

(b) Variable paroxysmal spontaneous attacks or exacerbation of pain, which frequently very.

Painful to patient; both (a) and (b) types are stimulus in dependent and described as shooting, stabbing or electrical.

(c)Stimulus-dependent pains reflecting the hypersensitivity of nervous system to many external stimuli such as touch, blunt pressure, hot or cold temperature, anxiety and even excitement.

(d) Paradoxical burning sensation in neuropathic pain: One cannot agree that paradoxical burning sensation occurs in subjects whose neuropathic pain is of central origin; it seems to occur somewhat randomly in a variety of neuropathic pain conditions.

The comprehensive examination of neuropathic pain patients should make use of sensory, motor and autonomic signs to confirm or reject the suspected anatomical localization of the lesion extracted from a careful history.

Table-1 Definition and assessment of negative and positive symptoms or sign in neuropathic pain.

SYMPTOMS/SIGN	DEFINITION	ASSESSMENT	EXPECTED PATHOLOGICAL RESPONSE
Negative sign and symptoms			
Hypoesthesia	Reduced sensation to non-painful stimuli	Touch skin with painter's brush, cotton swab or gauze	Reduced perception,numbness
Pallhypoesthesia	Reduced sensational vibration	Apply tunning fork to bone or joint	Reduced perception threshold
Hypoalgesia	Reduced sensation to painful stimuli	Prick skin with single pin stimuli	Reduced perception, numbness
Thermohypoesthesia	Reduced sensation to cold or warm stimuli	Touch skin with object of 10c, glass of water, coolants	
Spontaneous sensation/pain			
Paraesthesia	Non-painful ongoing sensation	Grade intensity(0-10) area in cm^2	-
Paroxysmal pain	Shooting electrical attacks foe seconds	Number per episode Grade intensity(0-10)	-
Superfacial pain	Painful ongoing sensational often of burning quality	Grade intensity(0-10) area in cm^2	-
Evoked pain			
Mechanical allodynia	Normally non-painful light pressure moving stimuli on skin evoke pain	Stroking skin with painter's bursh, cotton swab or gauze	Sharp burning superficial pain in the primary afferent zone, spreading in to unaffected skin areas
Mechanical static allodynia	Normally non-painful gentle sataic pressure stimuli on skin evoke pain	Manual gentle mechanical pressure to the skin	Dull pain in the area of affffected zone, primary afferent nerve ending
Mechanical punctuate or pinprickinhyperalgesia	Normally stining-but-not painful evoked pain	Manual pricking of the skin with a safety pain, sharo stick or stiff von frey hair	Sharp superficial pain in the primary affected zone, spreading in to unaffected skin areas
Cold allodynia	Normally non-painful cold stimuli evoked pain	Touch skin with object of 20c	Painful, often burning, temperature sensation in the area of affected primary afferent nerve ending
Heat allodynia	Normally non-painful stimuli evoked pain	Touch skin with object of 40c	Painful, burning, temperature sensation in the area of affected primary afferent nerve ending
Mechanical deep somatic allodynia	Normally non-painful pressure on deep somatic tissue evoked pain	Manual light pressure at joints or muscle	Deep pain in the joint or muscle

Causes of Neuropathic Pain

Neuropathic pain is a complex entity. It includes pain associated with damage to peripheral nerves (peripheral neuropathic pain), spinal roots (radiculopathic pain) and to the central nervous system (central pain). Various causes of neuropathic pain are listed in table 2. For differential diagnosis it is convenient to group the neuropathies into "asymmetrical" (nerve compression, neuroma, plexus neuropathies, etc.)

or "symmetrical" (metabolic-diabetes, pellagra; toxic-ethanol, INH; immune mediated. Phantom limb, cancer, failed back surgery syndrome (FBSS), diabetes, metabolic disorders, post herpetic neuralgia and stroke may all result in neuropathic pain. Approximately 8% of patients with stroke have central pain, whereas 10% to 30% of patients with spinal cord injury are affected by neuropathic pain. There are no data on the number of patients who have neuropathic pain from small fiber neuropathies, radiculopathies, brachial or lumbosacral plexopathies, complex regional pain syndrome or inflammatory peripheral conditions. Such pain is often severe, delayed in onset after the noxious event, shooting, lancinating or burning in quality and manifests even in the absence of an ongoing primary source for the pain. An area of sensory loss, either partial or complete, is an essential component of neuropathic pain.

Table-2 Causes of Neuropathic Pain (24)

Peripheral nerve	Spinal cord	Brain
Neuropathies	Traumatic injury	Stroke
Amputations	Vascular lesions	Multiple sclerosis
Traumatic injury	Multiple sclerosis	Syringobulbia
Entrapments	Avulsion injuries	Parkinson's disease
Herpes zoster	Syringomyelia	Epilepsy
Neoplastic invasion	Prolapsed disc	Tumors
Nerve disorder	Arachnoidities, tumors	Abscesses

Mechanisms of neuropathic pain

Neuropathic pain is thought to result when sensory neurons generate impulses at abnormal (ectopic) locations, for example at sites of nerve injury or demyelination. In the peripheral nervous system, in addition to firing spontaneously, these ectopic pacemaker sites are often excited by mechanical forces applied to them during movement. The result is spontaneous and movement-evoked pain. Damage to the CNS, such as in stroke or trauma, may cause ectopic firing of central origin or render brain circuits hyper excitable. In the light of the ectopic pacemaker theory, ectopic afferent firing is a primary source of spontaneous pain; it initiates and sustains central sensitization that manifests clinically as neuropathic hypersensitivity. In summary, today it is clear that numerous changes both central and peripheral occur in progression of nerve injury (24). The most important pathophysiological mechanisms known to play a role in neuropathic pain-

Peripheral Mechanisms of neuropathic pain

Since 1872 when Mitchell first provided a systematic description of various kinds of posttraumatic neuropathic pain as seen and treated by him in Philadelphia, extensive research on patients suffering from neuropathic pain and animal models of various neuropathies has resulted in major progress in the understanding of the basic mechanisms of neuropathic pain.

(a) Ectopic hyper excitability and abnormal sodium channels

Under normal condition, a noxious stimulus such as chemical, thermal and mechanical noxious stimuli activates the nociceptor by depolarizing the membrane of the sensory ending and triggering an action potential. Without the input of a stimulus, it is rare for normal primary afferent neurons to reach firing threshold. However, following a peripheral nerve injury, electrophysiological recording shows ongoing activity, abnormal excitability and discharge characteristics in the afferent nerve and neurons linked to the injury site and these Pathophysiological impulses have been demonstrated to originate from both injury sites and the related dorsal root ganglia (DRG) (22).This phenomenon has been termed ectopic discharge and also has been described in humans suffering from neuropathic pain (25).

Spontaneous impulses can be recorded few hours after transection of an axon and decreases with time. There is a positive correlation between such ectopic activity and the neuropathic pain behavior in animal models of neuropathic pain. The cause of ectopic discharges is still unknown. Changes in ion channel expression, localization, or biophysical properties may underlie the onset of ectopic discharges following peripheral nerve injury.

(b) Immune modulation of neuropathic pain

After peripheral nerve injury immune system attack on peripheral nerves or even simply immune activation near peripheral nerves can increase peripheral nerve hyper excitability and damage. Immune modulation is considered a significant contributor to neuropathic pain. The peripheral nerve contains a collection of immunologically relevant resident cells, including a large population of resident macrophages and numerous mast cells, dendritic cells, fibroblasts (26), Schwann cells, as well as the endoneourial capillary endothelial cells in the endoneurium. Upon activation, each of these cell types releases proinflammatory cytokines (tumor necrosis factor, interleukin-1, and interleukin-6), nitric oxide (NO), reactive oxygen species (ROS), and chemo attractant molecules that recruit immune cells to the site (27). Activated macrophages from recruited

and resident sources also release proinflammatory cytokines (28) further aggressive the damage.

Central mechanisms of neuropathic pain

The development of chronic pain following peripheral nerve injury has been attributed to the occurrence of three different processes in the spinal cord. These are the structural reorganization of cells, increased excitability, and decreased inhibition (29).

(a) Spinal cord -- Re-organization

Re-organization of the spinal cord in response to peripheral nerve injury has been demonstrated. The direct consequence of this re-organization is that secondary neurons within the spinal cord, which normally and predominantly receive high threshold sensory input from Ad- and C-fibers, begin to receive input from low threshold mechanoreceptors through Aß-fibers. This may result in low threshold sensory information being interpreted as nociceptive. Therefore, after peripheral nerve injury this re-organization in the spinal cord may contribute to the appearance of allodynia. This has been supported by electrophysiological studies. Lamina II cells in normal transverse spinal cord slice from rat exhibit long- latency responses to high threshold nerve stimulation.

After sciatic nerve section, 54% of the activity in lamina II is initiated by low-threshold stimulation and exhibited short latency response like those in lamina III of normal rats. However, CNS sprouting occurred only 2 weeks post injury, later than the appearance of allodynia observed in neuropathy models. The mechanisms for CNS sprouting have been investigated; including a role by neutrophins. NGF, but not NT-3 or BDNF, is able to prevent the sprouting of the Aßfibres into lamina II (30). NGF supplied retrogradly to the cell body is necessary to maintain the normal laminar distribution of terminals within the spinal cord.

(b) Spinal cord – hyper excitability

Evidence from a number of laboratories has repeatedly demonstrated that dorsal horn neurons including spinothalamic tract neurons can be 'sensitized' following activity in nociceptors. This 'sensitized' response includes a reduction in the threshold, and increase in the responsiveness, the expansion of the extent and the recruitment of novel inputs to receptive fields. Central sensitization of dorsal horn neurons in the spinal cord develops following peripheral nerve injury (31).

In the CCI model, about 90% of dorsal horn neurons exhibited abnormal characteristics (32), In including responses to very gentle mechanical stimulation of the nerve injury site, absence of peripheral receptive fields, and very pronounced spontaneous activity. The mechanism of central sensitization after peripheral nerve injury is unknown. Peripheral nerve lesions, and could result from pathophysiological pain stimuli and prolonged pain conditions (33). Such LTP might be involved in the development of behavioral hyperalgesia observed in rats following nerve lesions. There is also ample evidence to suggest that the excitatory amino acid glutamate and the neuropeptide substance P (SP) play important roles in the generation of central sensitization following peripheral nerve injury.

Role of Oxidative Stress in Neuropathic Pain

Oxidative stress may cause cell damage through increased oxidant generation, decreased antioxidant protection, and/or failure to repair oxidative damage. Cell damage is induced by reactive oxygen and nitrogen species (RONS), which are either free radicals, reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or be chemically activated by them (4). Under normal conditions, RONS are cleared from cells by the action of superoxide dismutase, catalase or glutathione, as well as antioxidant vitamins C and E. In pathological conditions, however, intracellular RONS level can cause severe cell damage and even cell death.

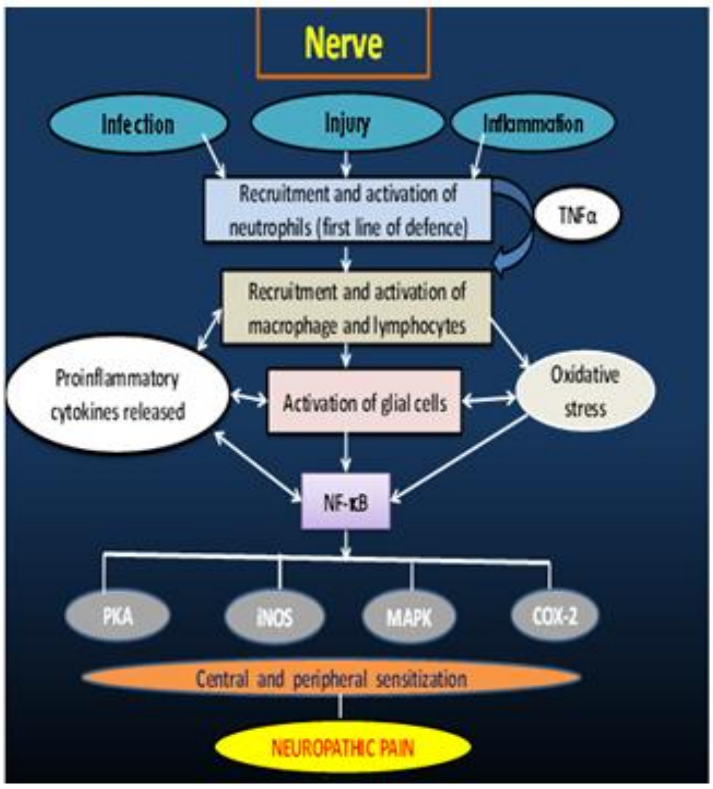

Fig-1 Pathophysiology of Neuropathic Pain (22)

Physiological concentrations of cellular ROS are tightly controlled by an endogenous antioxidant system which includes various enzymes and non-enzymatic molecules.

some pathological conditions, however, cellular ROS levels may rise beyond the normal physiological ranges, and thus lead to oxidative stress. This can be resulted from either increased ROS production or decreased antioxidant capacity. The consequences of oxidative stress vary from the modification of cellular signaling pathways to irreversible structural and/or functional damage. It has been reported that several mediators and neurotransmitters participate in central sensitization. Reactive oxygen species (ROS) seems to be involved in this concern, since it was shown that neuropathic pain reduces antioxidant activity in the spinal cord (34). In addition, systemic or intrathecal injections of antioxidants relieve allodynia (5). Thus, increasing evidence suggests a role for oxidative stress in neuropathic pain and in many neurological diseases, but the precise mechanisms that underlie pain and oxidative stress are still unclear. Increased production of ROS leads to marked changes in cell structure and function, as lipid peroxidation, protein oxidation and DNA damage some markers are useful to detect increased ROS, such as lipid peroxidation products. Hydrogen peroxide (H_2O_2) is a stable ROS that can be easily measured providing evidence of oxidative stress occurrence. The cell activity constantly produces superoxide anion (O2 •-) from molecular oxygen. This anion can be toxic if it reacts with other molecules such as nitric oxide (NO), but antioxidant enzyme superoxide dismutase (SOD) converts the superoxide anion to H_2O_2 (6). The damaging potential of H_2O_2 consists in its ability to yield hydroxyl radical (•OH) by the iron catalyzed Fenton and Haber–Weiss reactions. Thus, the increased H_2O_2 concentration may be harmful to cells. Moreover, H_2O_2 is a diffusible ROS contributing to the development of pathological pain states not only by generating dangerous ROS but also by modulating synaptic plasticity. This ROS play a role in releasing intracellular stored calcium and interfering in synaptic activity at dorsal horn interneurons.

In the chronic constriction injury (CCI) model of rat neuropathic pain, it has been shown that oxidative stress as well as the antioxidants superoxide dismutase and reduced glutathione are important determinants of neuropathological and behavioral consequences (35). Heat hyperalgesia is reduced by systemically injected antioxidants (7). However, one should realize that other studies in the CCI model observed (indirect) signs of inflammation also in nonneural tissues. Among these are increased skin blood flow and skin temperature at an early stage after nerve injury as well as edema and increased extravasation of polymorphonuclear leukocytes in muscle tissue (36). These inflammatory changes may also involve oxidative stress.

Role of Nitric Oxide in Neuropathic Pain

Nitric oxide (NO) has drawn the interest of numerous investigators since NO was identified as an endothelium derived relaxing factor (EDRF) (8). It has been shown that NO is synthesized from L-arginine by nitric oxide synthase (NOS) which is distributed in a variety of mammalian tissues and that NO functions as an intercellular messenger or neurotransmitter (37).Recent data from animal studies indicate a potential role for NO in maintaining chronic painful states in animals after lesions of peripheral nervous system and CNS (38). NO plays an important role in the maintenance of the behavioral signs of neuropathic pain and is involved in common steps in the maintenance of the different modalities of pain such as mechanical allodynia and cold allodynia (39).

It has been reported that intrathecal administration of NOS inhibitors reverses thermal hyperalgesic responses in rats after sciatic nerve ligation (40). There are also some reports that intracerebroventricular injection of L-arginine produces a biphasic effect on nociception, and NOS inhibitors have antinociceptive effects (41).NO is known to rapidly react with free radicals, namely superoxide anions, to form the stable but highly toxic peroxynitrite anion. The cytotoxicity of peroxynitrite is likely to dominate with progression of neuropathic pain, due to the excess availability of free radicals. This situation may be exacerbated by nerve injury and could contribute to delayed recovery. Hence we raised the possibility that excess free radicals and neuronal nitric oxide (nNO) generated with inflammation could contribute to the injury of nerves in rats. NMDA receptor activation in different regions of central nervous sytem (CNS) has been shown to result in a Ca2 +-dependent increase in cyclic guanosine monophosphate (cGMP) through the production of NO (42). NMDA receptor activation increases intracellular Ca2 + which activates NOS to produce NO from free L-arginine (43). NO a retrograde messenger has been suggested to either promote or reduce synaptic transmission of nociceptive stimuli in spinal cord. This depends upon the amount of NO released in the synaptic cleft. NO facilitates cGMP formation via activation of soluble guanylatecyclase modifies activation of K+ channels and Ca2+ current. NO-cGMP pathway modifies several intracellular processes, including activation of protein kinases, ion channels and phosphodiesterases (8). 8-bromo cGMP an analogue of cGMP in high doses induces hyperalgesia due to activation of PKG, whereas in low dose

induces analgesia mediated by PKG-independent mechanism. Besides this MB an inhibitor of a soluble guanylatecyclase decreases the production of cGMP induced by NO in the cell and plays an important role in development of hyperalgesia following the nerve injury. At rest, nNOS is expressed in some inhibitory interneurons and in <5% of primary afferent neurons. During inflammatory and neuropathic pain, nNOS is activated and upregulated in inhibitory interneurons and in primary afferent neurons, respectively (44). The increased NO production leads to activation of NO-GC and subsequent cGMP production in NO-GCexpressing neurons (45).

Role of Microglia in Neuropathic Pain

Glial cells (microglia and astrocytes) in the central nervous system (CNS) respond to the peripheral insults. As determined by up regulation of glial cell markers, both microglia and astrocytes are activated following peripheral nerve injuries such as nerve transection, ligation, and crush (46), and chemical insult (47). The glial cell activation could simply reflect the pathological responses elicited by peripheral nerve injury, as with glial cell responses in CNS induced by insults to brain or spinal cord (48).

However, given that glial activation following peripheral sensory nerve injury is directly correlated with pain facilitation such as hyperalgesia and allodynia observed in these animals (3), glial cell activation might play a more active physiological role, rather than simply reflecting these pathologic conditions (22). Indeed, a wealth of studies demonstrates the positive correlation between spinal glial activation and the development or maintenance of pain hypersensitivity (49). Accordingly, the current lack of appropriate treatment of neuropathic pain might be attributable to the limited understanding on the role of glial cells in pathological pain (50).

Based on these findings, the detailed following peripheral sensory nerve injury is directly correlated with pain facilitation such as hyperalgesia and allodyniaobserved in these animals (3), glial cell activation might play a more active physiological role, rather than simply reflecting these pathologic conditions (22). Indeed, a wealth of studies demonstrates the positive correlation between spinal glial activation and the development or maintenance of pain hypersensitivity (49). Accordingly, the current lack of appropriate treatment of neuropathic pain might be attributable to the limited understanding on the role of glial cells in pathological pain (50). Based on these findings, the detailed mechanisms by which the interaction between glial cells and spinal neurons eventually induce pathological pain, which are clearly different from those of acute nociceptive pain processing, are now under extensive investigation (49).

Although clinical studies indicate that neuropathic pain is more frequently observed in the trigeminal system than at the spinal level, most current studies use the injury models of spinal sensory nerve rather than those of orofacial regions (51). Several animal models with injuries to the infraorbital nerve or inferior alveolar nerve have been demonstrated to exhibit mechanical or thermal hypersensitivity. However, the involvement of glial cell activation in the pain hypersensitivity in these animals is still yet to be determined.

Role of COX and LOX pathways in neuropathic pain

A) Cyclooxygenase (COX) Pathway

COX, which converts arachidonic acid to endoperoxide-containing intermediates to produce prostaglandins and thromboxanes, exists in multiple isoforms. COX-1 and COX-2 share 60% amino acid sequence homology. Both enzymes are membrane bound; however, COX-2 is twice as abundant at the nuclear envelope as within the endoplasmic reticulum, whereas the concentration of COX-1 is equal at both locations.COX-2 is known to be up regulated during inflammation; however, Ma and Eisenach (have demonstrated (primarily in infiltrating macrophages) that COX-2 is universally upregulated following various types of peripheral nerve injury.

The resulting overproduction of prostaglandins appears to contribute to the central plasticity and maintenance of neuropathic pain after nerve insult, due, in part, to facilitat-ing the release of nociceptive neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), from primary afferent fibers with increased spinal dynorphin.The development of allodynia associated with neural insult may be partly due to spinal prostaglandins synthesized by COX-1 as well as COX-2. This finding is in contrast to the predominant role of COX-2 in inflammatory pain. Hefferan and colleagues found that spinal prostaglandins synthesized by COX-1 in the early period (4–8 hours) after neural insult appear to be important in the development of allodynia in rats.

These researchers compared the effects of intrathecal administration of SC-560 (a selective COX-1 inhibitor) with that of S(+)-ibuprofen (which inhibits both COX-1 and COX-221) and, since ibuprofen was better at reversing allodynia than SC-560, suggested that both COX-1 and COX-2 are important in the neuropathic pain that may emerge after neural insult. In much of the peripheral nervous system, COX-1 is expressed constitutively; peripheral COX-

2 is expressed to any physiologically significant degree only after tissue injury is induced secondary to inflammation.

However, the majority of neurons and radial glia in the spinal cord (ie, the glia in the white matter, not gray-matter glia, such as microglia astrocytes and oligodendrocytes) express significant, detectable levels of constitutive COX-2 .The constitutive COX-2 in the spinal cord is important in generating PGE_2, leading to hyperalgesia.blocking constitutive spinal COX-2 before tissue injury may diminish sensitization (both peripheral and central) following tissue injury. spinal prostanoids generated via both the COX-1 and COX-2 pathways may play a role in the hyperalgesia/allodynia seen in nerve-injured rats. Zhu and Eisenach working with partial sciatic nerve transsection and L5-L6 spinal-nerve ligation models in male rats, suggested that COX-1 expression in the spinal cord is not static and changes, in a time- and laminar-dependent manner, after nerve injury.

Further, these authors suggested that spinally administered specific COX-1 inhibitors may be useful in preventing and treating neuropathic pain. Ma and Eisenach provided morphological and pharmacological evidence of the role of peripheral prostaglandins in the pathogenesis of neuropathic pain. At 2 and 4 weeks following partial sciatic nerve ligation (PSNL), dramatic increases in COX-2 immunoreactive cell profiles were observed at the injured site and in the adjacent region. In addition, an increased number of COX-1 immunoreactive cell profiles were observed in the epidermis of the ipsilateral foot pad of PSNL rats. Local injection of ketorolac, a nonselective COX inhibitor, into the ipsilateral plantar side or into the injured region of the sciatic nerve reversed the mechanical allodynia induced by PSNL for more than 5 days and suppressed the PSNL-induced increase in the phosphorylation of a transcription factor cAMP (cyclic adenosine monophosphate) response element-binding protein in the ipsilateral spinal cord dorsal horn of L4 and L5. Broom et al reported similar results using the rat spared nerve injury model of neuropathic pain. They concluded that although COX-2 plays a key role in the development of inflammatory hypersensitivity induced by injection of complete Freund adjuvant into the rat hind paw, the pain hypersensitivity produced by this model is not significantly COX-2 dependent.

The clinical implications of these findings translate into the appropriate selection of selective COX-2 inhibitors for inflammatory pain—preferably one that crosses the blood-brain barrier reasonably well. However, in the setting of neural insult, it would appear reasonable to utilize agents that inhibit both COX-1 and COX-2 in order to diminish and/or prevent neuropathic pain. If this work is validated in human studies, clinical correlates may translate into the preferred use in certain subpopulations of traditional nonsteroidal anti-inflammatory agents that inhibit both COX-1 and COX-2 (perhaps with partial COX-1 selectiveness over COX-2) in situations where the potential for the development of neuropathic pain exists (eg, before treatment with vincristine).

B) Lipoxygenase (LOX) Pathway

In human tissues, arachidonic acid can be metabolized via three major lipoxygenases: 5- lipoxygenase (5-LOX), 12-lipoxygenase (12-LOX), and 15-lipoxygenase (15-LOX).Two isoforms of 15-LOX exist: 15-LOX type 1 and 15-LOX type 2. The two isoforms may have antagonistic effects, with type 1 providing anti-inflammatory effects, protection against bone loss, and perhaps analgesic effects via reduced expression of various cytokines (eg, interleukin 1β, tumor necrosis factor, and growth factors) and type 2 yielding opposing effects (eg, aggravating bone loss) (52). Of all the LOX isoforms, 5-lipoxygenase (5-LOX) catalyzes the initial steps in leukotriene biosynthesis (46). These leukotrienes can elicit most of the symptoms associated with inflammatory events including pain. Leukotriene B4 (LTB4) has been detected in samples of patients complaining hyperalgesia during masseter muscle and radicular pain. Hyperalgesia occurs following LTB4 challenge through different routes.

Furthermore, LTB4 decreases the mechanical and thermal threshold of nociceptors. Recently, the role of lipoxygenase metabolites in prostaglandin and epinephrine-mediated mechanical hyperalgesia.PGs and LTs have complementary effects in the development and persistence of inflammatory pain. It is widely recognized that in states of pain following tissue injury, prostaglandins generated locally act to sensitize peripheral nociceptors to noxious stimuli and subsequently release other mediators in spinal cord resulting in hyperalgesia (53).

There is evidence that leukotrienes (LTs) at the peripheral and spinal level also exert similar effect. Studies in animals and humans have demonstrated that LTs, the products of NSAIDs resistant 5-LOX-catalyzed metabolism of AA, also produce hyperalgesia. Peripherally, LTs have been shown to possess similar effect as PGs in regulating hyperalgesia (54). Recently,(55) reported the central effect of LTs in modulating hyperalgesia in formalin test in mice.

3. Pain behavior in animals following nerve injury

Neuropathic pain is a challenging problem, which

is considered to be particularly difficult to treat. The study of the mechanism of neuropathic pain is largely based on animal models. Animals undergo nerve lesions which are clinically relevant and behavioural experiments are performed before and after the surgical interventions. The signs of pain are measured by quantifiable behavioural components such as paw withdrawal latency to thermal stimulation and paw withdrawal threshold to mechanical stimulation with von Frey hairs. These reactions are interpreted as being equivalent to thermal and mechanical allodynia in humans. The quantitative data obtained in behavioural experiments guide the design of further reduced animal models which can offer an insight into the neuronal mechanisms of sensory perception. Development of animal models that reflect elements of clinical pain syndromes has led to advances in understanding the pathophysiological mechanisms of initiation and maintenance of neuropathic pain.

One important drawback of the animal models of neuropathic pain is the lack of verbal communication in the animals. Thus, the measure of pain in animals is largely based on some empiric behavior responses. To find more effective way to manage neuropathic pain clinically, a large number of experimental studies have been carried in animals. Different animal models are used to assess neuropathic pain. These includes-

1) Total nerve transaction and ligation

Simulating the clinical conditions of amputation.

2) Partial nerve lesion (PNL)

PNL with a tight ligation around a portion (about 50%) of the nerve fascicles, simulating the clinical condition of an accidental nerve bruise or gunshot-induced nerve injury.

3) Chronic constriction injury(CCI)

CCI by placing several loose ligatures around the nerve, leaving a lumen of less than the diameter of the original nerve, simulating the clinical condition of chronic nerve compression such as the one that occurs in nerve entrapment neuropathy or spinal root irritation by a lumbar disk herniation.

There are three behavioral indices of neuropathic pain which are often seen in animals with a partial nerve lesion or a peripheral or spinal nerve constriction injury:

Autotomy

A form of excessive self-care or self- grooming occurs that results in bite wounds and eventually in the self-amputation of digits.

Hyperalgesia

A stronger or earlier withdrawal response than in healthy animals to a noxious stimulus, e.g. noxious skin heating.

Allodynia

Withdrawal from a stimulus that is not painful in tests on humans and does not evoke withdrawal in animals without nerve injury. The most common form is mechanical allodynia, cold allodynia has also been described.

Tight ligation of a spinal nerve (SNL)

SNL or transaction of one or several dorsal roots (22) resulting in complete deafferentation of one or several spinal segments, simulating nerve plexus and dorsal root injury.

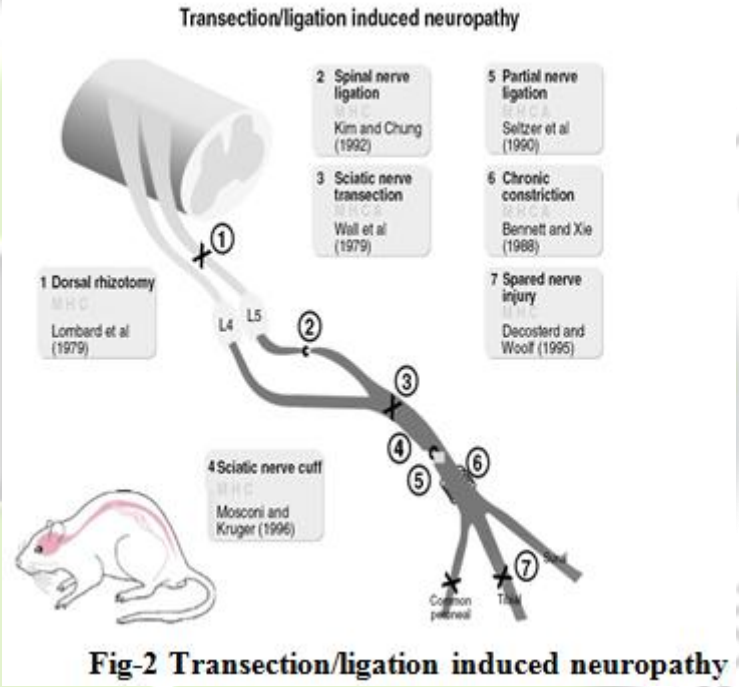

Fig-2 Transection/ligation induced neuropathy

Schematic showing the various animal models of neuropathic pain.Models of peripheral nerve injury by ligation or transaction. The diagram indicates the sciatic nerve from peripheral trifurcation to dorsal roots (region indicated in lower left corner). The incidence of neuropathic pain in each model is indicated by the key: M, mechanical allodynia; H, thermal hyperalgesia; C, cold allodynia**(**56)

7) Spared nerve injury (SNI)

Decosterd and Woolf (2000) developed a new animal model which consists of ligation and section of two branches of the sciatic nerve (namely tibial and common peroneal nerves) sparing the sural nerve (Spared Nerve Injury [SNI] model).The SNI model results in early (less than 24 hours), prolonged and substantial changes in mechanical and cold sensitivity that closely mimic the features of clinical neuropathic pain. The SNI model differs from the SNL model in that the intermingling of intact and injured neurones in peripheral target tissues is restricted to the border territory between the lesionedtibial nerve and intact sural nerve. This permits behavioural testing of the non-injured sural nerve territory (adjacent to the denervated.

areas) and also enables to investigate the neurophysiological changes which take place in the peripheral afferent terminals of the intact sural afferent neurones following injury of neighbouring nerves.

4) Spinal nerve ligation (SNL)

The spinal nerve ligation model (SNL) consists of a tight ligature of one (L5) or two (L5 and L6) spinal nerves close to the dorsal root ganglion, leaving the L4 component of the sciatic nerve intact. The L5 spinal nerve lesion leads in rats to an early onset and long-lasting mechanical and thermal allodynic behaviour. The mechanical allodynic behaviour is supposed to be triggered and maintained by ectopic activity generated in lesioned afferent neurones. Three to 8 days and 20 to 53 days after L5 spinal nerve lesion spontaneous discharges were present in 56% and respectively 29% of the L5 dorsal root filaments, spontaneous activity being observed in afferent neurones with myelinated fibres but not in those with unmyelinated nerve fibres. A temporal but weak correlation between ectopic activity and behaviour was found suggesting that the ectopic activity in lesioned afferent neurones might be important for the maintenance of the neuropathic pain behaviour.

Studies conducted in the same laboratory by demonstrated that mechanical allodynic behaviour which develops in rats after L5 spinal nerve lesion did not disappear after transection of the dorsal root L5, suggesting that besides the spontaneous activity in the lesioned afferent nerve fibres there is another mechanism which account for the abnormal reactions to mechanical and thermal stimuli in rats with SNL. the abnormal pain behaviour which develops in rats after lesion of the L5 spinal nerve might be dependent on ectopic activity in intact (non-lesioned) afferent neurones. Recordings made from C-fibres in the intact L4 spinal nerve after ligation and transection of the L5 spinal nerve showed that one day after lesion, spontaneous activity developed in approximately half of the afferent C fibres. The alterations in the properties of intact L4 afferent neurones might be due to their interaction, distal to the lesion site, with the products associated with degeneration of the lesioned L5 neurones.

5) Nerve lesion followed by regeneration

When a nerve is lesioned and the regeneration to the periphery is prevented (e.g., neuroma lesion) the injured myelinated and unmyelinatedfibers develop ectopic spontaneous and evoked activity. Studies done on rats, showed that 6-30 hours after sural nerve ligation and section about 18% of the axotomizedunmyelinated and about 30% of the myelinated nerve fibres developed ectopic activity. The unmyelinated units became responsive to mechanical, cold or heat stimuli and could develop spontaneous activity. The myelinated afferents developed sensitivity to mechanical stimuli applied to their novel axon endings and only few of them had spontaneous activity. In some clinical situations after peripheral nerve injury the lesioned afferent nerve fibres are free to regenerate to the peripheral target tissues. In this case, the cut distal end of the lesioned afferent nerve fibres send sprouts which tend to reach the target tissues. Growth factors synthesised by the non-neuronal cells post nerve injury are believed to be responsible for the sprouting of the lesioned fibres towards the target tissues. Following nerve lesion, cytokines (e.g. interleukines, IL; tumor necrosis factor, TNF ") are produced and released by Schwann cells during Wallerian degeneration in the distal stump of the nerve. The intimate contact of the lesioned and regenerating nerve fibres with the rich cytokine environment distal to the lesion site may influence the excitability of the injured myelinated and unmyelinated fibres during the regeneration process. The pattern and distribution of the abnormal discharge properties of the lesioned fibres can be different depending on the type of lesioned afferent fibre (A- or C-fibre), on the type of nerve lesion and can vary in time during the regeneration process and after the axons have reinnervated the target tissues. The change in the characteristics of spontaneous and evoked activity influences the status of excitability in spinal nociceptive neurones and consequently the sensory abnormalities associated with neuropathic pain.

Other animal models have also been developed to mimic individual disease states, such as the streptozotocin model of peripheral diabetic neuropathy (58), a CCI of the infraorbital branch of the trigeminal nerve as a model for trigeminal neuralgia (59), and the model of acute herpes zoster for acute zoster and post-herpetic neuralgia (60).

Behavioural Testing

Rats which underwent Spared Nerve Injury (SNI) were tested for signs of mechanical and thermal allodynia post nerve injury. In order to assess mechanical and cold sensitivity of the hind paws the rats were placed in transparent plastic boxes above a wire mesh floor which allowed full access to the paws. Animals were tested in groups of four. Behavioural accommodation was allowed for at least 10 min before testing started. All the behavioural tests were done on the hind paws both ipsi- and contra-lateral to the nerve lesion. The area tested was the medial plantar surface of the hind paws for the *tibial nerve territory* and lateral plantar surface of the hind paws for the *sural nerve territory* (Fig.3).

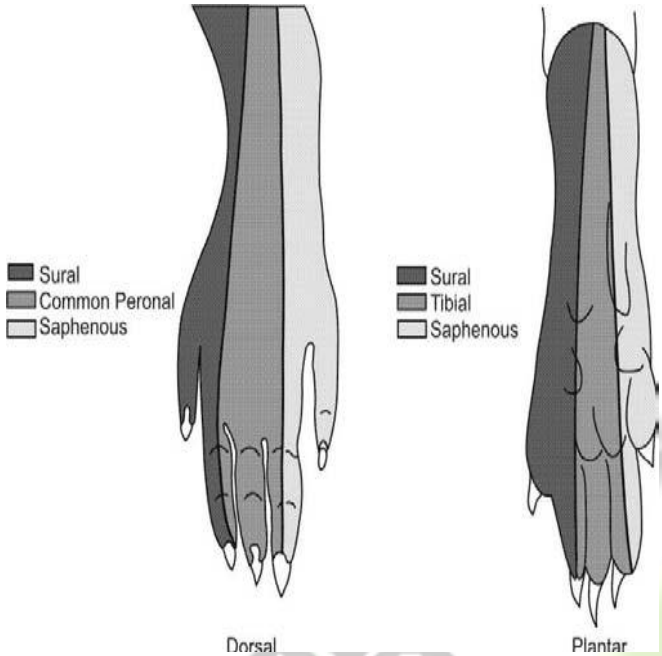

Fig-3 Different zones of the dorsal and plantar surfaces of the rat hind paw innervated by the three terminal branches of the sciatic nerve (sural, tibial and common peroneal nerves) and by the saphenous nerve.

Following SNI, the animals showed an abnormal paw exposure due to denervation produced by lesion of the tibial and common peroneal nerves. Therefore, cold and heat sensitivity of the medial plantar surface of the hind paw (tibial nerve territory) ipsilateral to the lesion side could not be assessed. Standard testing procedures were used to quantify signs of mechanical and thermal allodynia. The baseline measurements were taken 3 times before nerve lesion. The rats were then tested on day 1 after lesion, twice a week until day 13 post operation and further on once a week until day 55 post nerve lesion.

3.2.1 Testing of mechanical sensitivity

Mechanical sensitivity was quantified using the up-down method (Fig. 11). An ascending series of von Frey filaments that delivered approximately logarithmic incremental forces (7.5 to 220 mN) were applied to the test area for about 8s. The 45 mN stimulus was applied first. Whenever a positive response to the stimulus (flexion reaction) occurred the next weaker von Frey hair was applied; whenever a negative response (no reaction) occurred the next stronger force was applied. The test was continued until: a) the response to 5 more stimuli after the first change in response had been obtained or b) the upper/lower end of the von Frey set was reached before a positive or negative response had been obtained. The pattern of positive and negative responses, respectively, was converted to a threshold value using the formula: threshold = $10^{(X+Kd)}$ where X = value of the final von Frey hair used (in log units); K = the tabular value for the pattern of positive/negative responses and d = mean difference in strength (in log units) between stimuli (here 0.183). In cases when continuous positive or negative responses to stimuli at either end of the stimuli range used were observed, values of either 5.8 mN (for continuous positive responses) or 271.9 mN (for continuous negative responses) were assigned. These numbers correspond to a von Frey hair 1 log increment below the smallest or 1 log increment above the highest von Frey hair used.

Fig-4 Testing procedure for mechanical sensitivity of the hind paws.

Testing of cold sensitivity

Cold sensitivity was quantified by measuring the duration of paw withdrawal in response to acetone application. An acetone drop was formed at the end of a piece of a small polyethylene tube which was connected with a syringe. The drop was gently applied to the medial or lateral plantar surface of the hind paws. The acetone was applied 5 times (once every 3-5 min) to each paw and the duration of paw withdrawal (in seconds) was measured.

Testing of heat sensitivity

Heat sensitivity was quantified using the Hargreaves test. Rats were placed in a transparent plastic chamber with a glass floor (Fig. 12). Measurements of withdrawal latency to heat stimuli began after a period of accommodation of 10-20 minutes. A radiant heat source consisting of a high intensity projector lamp bulb (Osram 58-8007, 8V, 500W) was focused on the test territories of the hind paws. Onset of the radiant stimulus triggered a timer which was stopped by subsequent paw movement. Five heat stimuli were delivered to the lateral plantar surface of the ipsi- or contra-lateral hind paws at 3-5 min intervals. Following SNI the rats did not place the entire foot on the glass floor. Every attempt was made to focus the thermal stimulus on the sural innervation

territory that was in full contact with the glass floor since it was reported that the withdrawal latency (which is related to the heat transfer) may vary with the degree of paw contact.

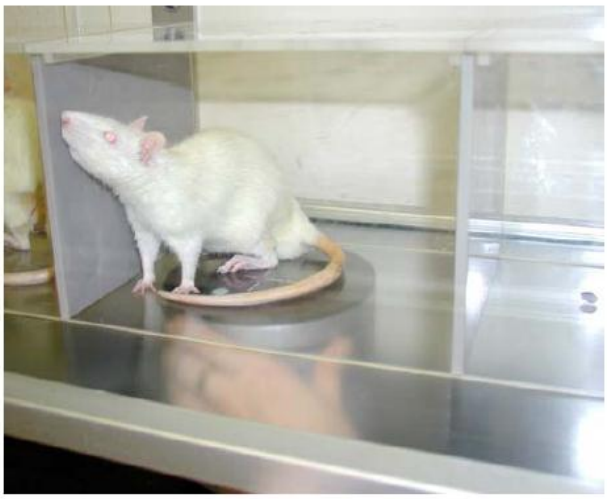

Fig-5Testing procedure for heat sensitivity of the hind paws

For a given test day the sequence of the above mentioned behavioural tests was: 1) mechanical stimulation 2) cold stimulation 3) heat stimulation. The order of the tests was kept the same throughout the whole investigation period for all animals. The rationale for the choice of such order is that the least stressful test was performed first to minimise the influence of one test on the result of the next one.

Management of Neuropathic Pain

The treatment of the underlying causative condition is central to the management of neuropathic pain but is outside the scope of this record. Neuropathy caused by mechanical pressure for example may require surgical and other interventional procedures.

Non-pharmacological measures

Psychological techniques - there are no meta-analyses in the management of neuropathic pain *per se*, but CBT performed well in patients who had chronic pain from various causes. The trials that have been done indicate that CBT and other psychological techniques may be helpful in some forms of neuropathic pain (61). Studies of chronic pain management suggest that a combination of psychological, pharmacological and physical therapies, tailored to the needs of the individual patient, may be the best approach.

Patient education - this is often quoted as a strategy, though the evidence-base is lacking. Logic would dictate however that an informed patient is more able to be involved in decisions about their care, and this is certainly relevant in terms of compliance (62)

Electrical stimulation - interpretation of systematic trials are difficult due to differing methodologies. However, transcutaneous electrical nerve stimulation (TENS) performs consistently well compared to placebo.

Acupuncture - systematic evidence to support its use in neuropathic pain is lacking. There is some evidence that percutaneous nerve stimulation (PENS), which combines the techniques of acupuncture and TENS, may be helpful for some patients with diabetic neuropathy.

4.2 Pharmacological measures

An important recommendation in initiating pharmacologic therapy for neuropathic pain is to introduce one drug at a time, with gradual upward titration, based upon the patient's response. Prescribing several agents at one time precludes determination of the most effective agent or, if side effects occur, the agent responsible for the complications.Most controlled trials have focused on the relief of pain, rather than the sometimes more distressing symptoms of allodynia or hyperalgesia, or the effect of treatment on the quality of life. This makes a comparative interpretation of the available treatments difficult. The general consensus, however, supports the use of the following drugs:

Non-opioid analgesics and NSAIDs

Non-opioids, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, have limited usefulness in the management of neuropathic pain (63). All NSAIDs have the same mechanism of action inhibiting the enzyme cyclooxygenase (COX) and consequently prostaglandin synthesis. By inhibiting prostaglandins NSAIDS reduce tissue inflammation and pain. NSAIDs mainly act peripherally, mostly NSAIDs are relatively non-selective, inhibiting both COX-1 and COX-2 enzymes in a ratio that varies from drug to drug The common side effects include gastrointestinal irritation and risk of gastrointestinal bleeding, renal toxicity. These perform well against placebo, particularly in diabetic neuropathy, but most patients require more targeted treatments (64). However, some patients do report relief, so a trial may be indicated. Many patients have concomitant neuropathic and nociceptive pain, which may respond to non-opioids.

The tricyclic antidepressants (TCAs) provide the best evidence of efficacy in the management of Neuropathic pain. A Cochrane review found that the 'numbers needed to treat' (NNT) for tricyclic antidepressants (TCAs) and venlafaxine was three (65). In other words, of every three patients prescribed these drugs, one would obtain at least moderate relief from neuropathic pain. There was no difference in the NNT between TCAs with balanced inhibition of reuptake of serotonin and noradrenaline (amitriptyline, imipramine) and those with relatively selective inhibition of noradrenaline

uptake (desipramine, nortriptyline). Similarly, in terms of the NNT, the efficacy for TCAs was nearly identical regardless of the underlying condition: diabetes mellitus, herpes zoster, traumatic nerve injury or stroke.

Although the definitive mechanism of action of tricyclic analgesia is unknown, these drugs block the reuptake of noradrenaline and serotonin, block hyperalgesia induced by N-methyl-D-aspartate agonists and also have sodium channel blocking properties. The TCAs, therefore, have analgesic properties independent of their antidepressant effects (66).

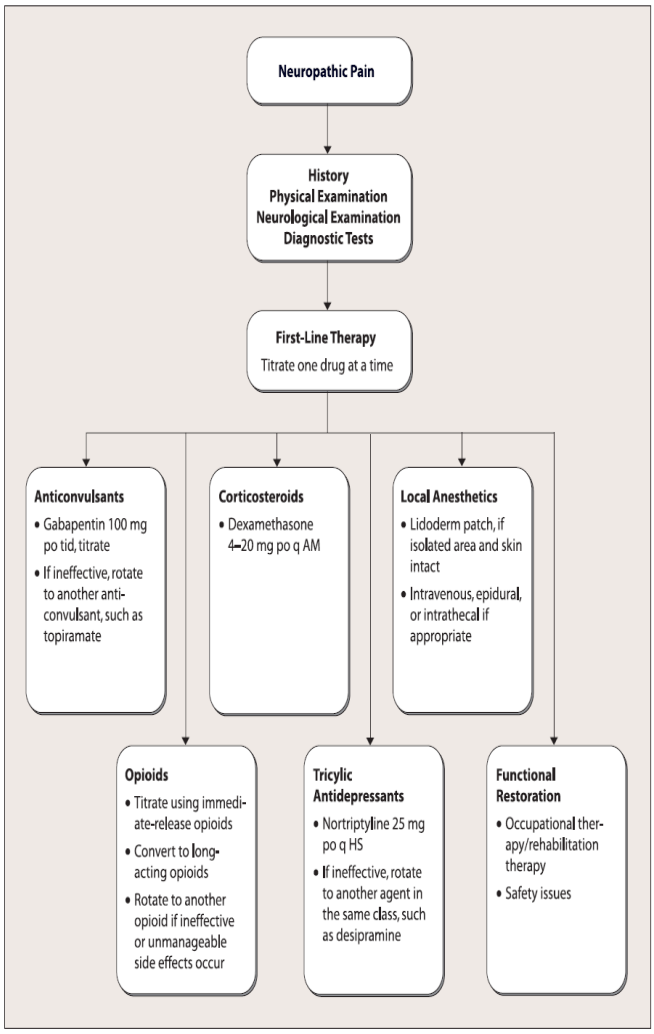

Fig- 6 Algorithm for Management of Neuropathic Pain

If these first-line therapies are ineffective, consider other agents, such as GABA-B agonists (baclofen) or NMDA antagonists (such as ketamine). Nerve blocks may be indicated in certain patients. Cancer therapies, such as radiotherapy, may be helpful to reduce tumor size and compression against nerve roots (63).

Amitriptyline is the most frequently prescribed TCA (unlicensed use), initially 10-25 mg nocte. The dose may be increased gradually to 75 mg daily if required. Higher doses should be instituted under specialist supervision. The anticholinergic effects of amitriptyline not are well tolerated, particularly in the elderly. Alternative agents, with fewer adverse effects, include nortriptyline (Aventyl, Pamelor) and desipramine (Norpramin, Pertofrane).

A systematic review showed no difference between the tricyclics regarding effectiveness, but nortriptyline, also given initially at 10-25 mg at night, may cause fewer side effects.Patients with pre-existing conduction abnormalities should have a baseline electrocardiogram, as the tricyclic antidepressants can alter cardiac conduction. In all patients, start at a low dose, usually at bedtime, and titrate every 3–7 days, based on the patient's response. Recent studies conducted in cancer patients demonstrate only slight analgesic effects from amitriptyline and nortriptyline (63).

Anti-convulsants

Older anticonvulsants, particularly carbamazepine, phenytoin, or valproate, have been used extensively to treat neuropathic pain, yet potential adverse effects require careful monitoring, particularly for neutropenia and megaloblastic anaemia. The newer anticonvulsant, gabapentin (Neurontin), approved for treatment of complex partial seizures, has been shown to have analgesic properties in both animal and human models of neuropathic pain. Two well-designed, randomized, controlled, multicentre studies evaluated the efficacy of gabapentin in postherpetic neuropathy and diabetic neuropathy (63). Both gabapentin and pregabalin are licensed for the treatment of neuropathic pain. Gabapentin and pregabalin bind to presynaptic voltage-gated calcium channels in the dorsal horn, resulting in a decrease in the release of excitatory neurotransmitters such as glutamate and substance P.

A Cochrane review supported the use of gabapentin in chronic neuropathic pain, but was inconclusive about its role in acute pain (*67*). In two studies of painful diabetic neuropathy and post herpetic neuralgia, gabapentin produced significant pain relief relative to placebo, and significant improvement in measures of quality of life and mood. The combined NNT for gabapentin in the management of Neuropathic pain is approximately 4.

A recent trial supported the use of pregabalin in central neuropathic pain. Pregabalin is an analogue of gabapentin with the same mechanism of action, but manifests linear pharmacokinetics and has higher affinity for the presynaptic calcium channel. Large RCTs have shown that pregabalin provides significant pain relief and improved quality of sleep in post herpetic neuralgia, painful diabetic neuropathy or both. The overall NNT for pregabalin in these conditions is 4.2. Pregabalin has also been studied in chronic central Neuropathic pain following spinal cord injury, with resulting

evidence of significant pain relief (66).

Both drugs can cause dizziness, drowsiness, and peripheral oedema. However, trials suggest that pregabalin is effective at lower doses, and therefore has less potential than gabapentin for causing adverse effects at a symptom-controlling dose.

Lamotrigine is a novel anticonvulsant agent that may act through voltage-gated cation channels to produce inhibition of glutamate release. Lamotrigine has been found to be useful in the management of trigeminal neuralgia and painful diabetic neuropathy. However, lamotrigine was not found to be useful in the management of a variety of peripheral Neuropathic pain states. Lamotrigine also requires slow and careful titration and carries a risk of Stevens-Johnson syndrome.

Topiramate and valproic acid have produced mixed results in Neuropathic pain trials.

Carbamazepine remains the drug of first choice for tic douloureux (idiopathic trigeminal neuralgia) but otherwise is not recommended for the management of neuropathic pain.

Levetiracetam is a novel anti-epileptic drug that binds to the synaptic vesicle protein SV2A in the brain and spinal cord. Despite the antinociceptive effect found in animal models of neuropathic pain, levetiracetam did not relieve post-mastectomy syndrome and spinal cordinjury pain (68).

Opioids

These may produce only a partial response, but may be useful where other methods fail. There is systematic evidence to support the use of tramadol and oxycodone (69). There are small randomized trials supporting the use of methadone, but larger systematic reviews are required. A Cochrane review found that whilst short-term trials were contradictory, intermediate-term trials provided evidence of effectiveness. It concluded that longer-term trials were required.

A recent systematic review of eight high-quality RCTs of up to eight weeks' duration demonstrated clinically important analgesia in Neuropathic pain state. Three trials involved morphine, three involved oxycodone, and single trials involved methadone and levorphanol. All these trials demonstrated significant benefit relative to placebo or a dose-dependent analgesic response. On average, these studies demonstrated a 20% to 30% reduction in pain intensity. RCTs in patients with post herpetic neuralgia given controlled-release oxycodone or controlled-release morphine showed a significant reduction in pain intensity, with variable improvement in sleep and disability. Trials of controlled-release oxycodone in painful diabetic neuropathy showed more consistent improvement in pain, sleep and ability to function relative to placebo. The NNT for morphine and oxycodone was approximately 2.5.

Tramadol is a unique analgesic agent that demonstrates low affinity binding for the mu opioid receptor, and inhibits reuptake of noradrenaline and serotonin. Tramadol is a weak opioid agonist and mimics some of the properties of the TCAs. Tramadol has shown significant benefit in three RCTs of painful diabetic neuropathy and mixed Neuropathic pain syndromes, and provides an overall NNT of 3.9. Tramadol produces less constipation and nausea than other weak opioid analgesics such as codeine, but is much more expensive.

Methadone is a synthetic opioid analgesic that may be useful in the management of Neuropathic pain because it has N-methyl-D-aspartate antagonist properties. Two small RCTs demonstrated benefit from methadone in chronic neuropathic pain and survey data suggested efficacy in mixed neuropathic pain conditions. Methadone has excellent oral bioavailability and duration of action of at least 8 h with repetitive dosing. However, it has an eliminationhalf-life of 24 h to 36 h, which requires close observationduring the titration phase. Because methadone ischallenging to titrate, lacks high quality evidence of efficacy and it is relegated to fourth-line status as ananalgesic for neuropathic pain.

The cannabinoids are analgesic agents with strong evidence of efficacy in animal models and increasing evidence of efficacy in Neuropathic pain states (66).

Table-3 Dosing regimens for selected agents for neuropathic pain

Agent	Starting dose and titration	Usual maintenance dose	Adverse effects	Comments
Tricyclic antidepressants				
Amitriptyline Nortriptyline Desipramine Imipramine	10–25 mg/day; increase weekly by 10 mg/day	50–150 mg/day	Drowsiness, confusion, orthostatic hypotension, dry mouth, constipation, urinary retention, weight gain, arrhythmia	Amitriptyline more likely to produce drowsiness and anticholinergic side effects; contraindicated in patients with glaucoma, symptomatic prostatism and significant cardiovascular disease
Serotonin noradrenaline reuptake inhibitors				
Venlafaxine	37.5 mg/day; increase weekly by 37.5 mg/day	150–225 mg/day	Nausea, dizziness, drowsiness, hyperhidrosis, hypertension, constipation	Dosage adjustments required in renal failure
Duloxetine	60 mg/day	60–120 mg/day	Sedation, nausea, constipation, ataxia, dry mouth	Contraindicated in patients with glaucoma; duloxetine not available in Canada
Anticonvulsants				
Gabapentin	300 mg/day; increase weekly by 300 mg/day	300–1200 mg three times daily	Drowsiness, dizziness, peripheral edema, visual blurring	Dosage adjustments required in renal failure
Pregabalin	75–150 mg/day; increase weekly by 50–150 mg/day	150–300 mg twice daily	Drowsiness, dizziness, peripheral edema, visual blurring	Similar adjustments in renal failure
Carbamazepine	100 mg once daily; increase weekly by 100–200 mg/day	200–400 mg three times daily	Drowsiness, dizziness, blurred vision, ataxia, headache, nausea, rash	Drug of first choice for tic douloureux (idiopathic trigeminal neuralgia); as an enzyme inducer, might interfere with activity of other drugs like warfarin; monitoring of blood counts and liver function tests recommended
Controlled-release opioid analgesics				
Morphine Oxycodone Fentanyl	15 mg every 12 h 10 mg every 12 h 25 μg/h patch	30–120 mg every 12 h 20–60 mg every 12 h 25–100 μg/h patch	Nausea, vomiting, sedation, dizziness, urinary retention, constipation	Constipation requires concurrent bowel regimen; addiction is unusual unless there is a past history of substance abuse
Others				
Tramadol	50 mg/day; increase weekly by 50 mg/day	50–150 mg four times daily	Ataxia, sedation, constipation, seizures, orthostatic hypotension	May lower seizure threshold: use with caution in epilepsy; in combination with acetamino-phen, keep maximal dose of acetaminophen at 4 g to avoid hepatic toxicity
Lidocaine		5% patches or gel applied to painful areas for 12 h in a 24 h period		Most useful for postherpetic neuralgia; has virtually no systemic side effects; lidocaine patches not available in Canada
Dronabinol	2.5 mg twice daily	2.5–10 mg twice daily	Dizziness, drowsiness, euphoria	Causes positive urine drug testing for cannabinoids
Tetrahydrocannabinol/ cannabidiol	1–2 sprays every 4 h, maximum four sprays on day 1, titrate slowly	2 sprays four times daily	Dizziness, fatigue, nausea euphoria	Conditionally approved for neuropathic pain associated with multiple sclerosis; causes positive urine drug testing for cannabinoids; monitor application site (oral mucosa)

Local anaesthetics

Local anaesthetics inhibit pain primarily by blocking sodium channels and are particularly useful in neuropathic pain syndromes. Lidocaine (Lidoderm) (5%) patches have been found to reduce pain related to post herpetic neuropathy, without any significant accumulation of drug in plasma levels after application of up to three patches. Lidocaine patch 5% has been shown to be useful in the management of a variety of focal Neuropathic pain syndromes, with an NNT of 4.4 (63). However, all of these trials were of short duration (up to three weeks) and had other limitations. One trial used an enriched enrolment design (only patients who responded to open-label treatment were included) and two other studies were derived from post hoc analyses of larger trials involving multiple neuropathic pain states new evidence from an open-label study suggests that lidocaine patches are useful, not only in post herpetic neuralgia or very localized neuropathic pain but also in painful diabetic neuropathy. Transient receptor TRPV1 agonists and antagonists represent a new class of analgesics. Lidocaine gel (5%) has demonstrated significant pain relief for up to 8 h in post herpetic neuralgia. Topical lidocaine is a safe treatment with no or limited systemic side effects (66).

Oral lidocaine analogues such as mexiletine (Mexitil) have been shown to be effective in some patients. However, mexiletine has produced positive results in only two of seven Neuropathic pain trials. Mexiletine is a class 1B local anaesthetic antiarrhythmic agent whose mechanism of action is blockade of sodium channels. Local anaesthetics suppress ectopic neural pacemaker sites at lower concentrations than required for conduction block along the nerve and therefore may have a prolonged duration of action.

Intravenous lidocaine infusions are gaining acceptance in a variety of pain-management settings, including pain clinics, hospices, and palliative-care centers. A bolus intravenous dose of lidocaine (1–2 mg/kg) is given over 15–30 minutes. If this is effective, it may be followed by a continuous infusion of 1–2 mg/kg/h. In some patients, the effects can be quite prolonged, giving up to weeks of relief. An early warning sign of potential toxicity is perioral numbness. An intravenous infusion of lidocaine 5 mg/kg over 30 min to 60 min may produce analgesia that lasts several hours or longer.

Selective serotonin reuptake inhibitors

The role of selective serotonin reuptake inhibitors (SSRIs) inthe management of Neuropathic pain is unclear. Citalopram andparoxetine have been found to be efficacious in themanagement of painful diabetic neuropathy independent oftheir antidepressant effects, but fluoxetine has not.However, the combined NNT for all three studies was 6.7 thus SSRIs do not appear to be as efficacious as TCAsor SNRIs. SSRIs used primarily for depression may inhibitthe metabolism of TCAs and increase the risk of serotoninsyndrome. Serotonin selective reuptake inhibitors, such as fluoxetine (Prozac), appear to have little efficacy in relieving neuropathic pain.

Serotonin noradrenaline reuptake inhibitors

The newer mixed serotonin noradrenaline reuptake inhibitors (SNRIs), venlafaxine and duloxetine, have NNTs of approximately 4.6 and 5.2, respectively. Duloxetine has demonstrated significant pain relief relative to placebo in three RCTs involving patients with painful diabetic neuropathy. Venlafaxine has shown efficacy in trials involving painful diabetic neuropathy and mixed painful polyneuropathy at doses of 150 mg to 225 mg per day. However, the latter trial also compared venlafaxine with imipramine, and imipramine showed a higher proportion of responders. Newer agents, such as venlafaxine (Effexor), a serotonin and norepineuropathicpainhrinereuptake inhibitor, may be more effective in ameliorating neuropathies in cancer patients. SNRIs are also effective in patients with cardiac disorders (DE Moulin *et al.*, 2007).

Topical creams

Capsaicin cream is licensed for neuropathic pain. It can be applied three to four times a day. It should not be used on broken or inflamed skin. It can cause irritation at the site of application (70). Systematic review suggests it may be useful as an adjunct for a small number of patients unresponsive to other treatment. A single application of a high-concentration capsaicin dermal patch produced sustained pain reduction in patients with post herpetic neuralgia and painful HIV-associated sensory polyneuropathy, although the long-term effect and the frequency of treatments have not been examined. NGX-4010 (marketed name Qutenza®), a cutaneous patch of **capsaicin 8%,** has been given marketing authorization in Europe. The approved indication is: "treatment of peripheral neuropathic pain in non-diabetic adults". NGX-4010 treatments may be repeated every 90 days (68).

Although there have been no randomized clinical trials, corticosteroids have long been used to treat a variety of neuropathic pain states, particularly those related to cancer. Corticosteroids are sometimes given to relieve pressure in compression neuropathy. Dexamethasone has the least mineralocorticoid effect and due to the long duration of effect, dosing can be scheduled once per day. This dosing

schedule fosters adherence and prevents sleep disturbances that may result from the stimulant effects of this drug when administered in the afternoon or evening. Unfortunately, immunosuppressant and endocrine effects limit long-term use. Proximal muscle wasting also can occur after 4–6 weeks of therapy (63).

Miscellaneous agents

Clonidine, an alpha2-agonist sympathetic blocker, showed benefit in a subset of patients with painful diabetic neuropathy in an enriched enrolment trial.

Recent advances in management

Botulinum toxin type A (BTX-A) given intradermally is another novel topical treatment approach that has been shown to relieve focal painful neuropathy and painful diabetic polyneuropathy in two RCTs. Patients received multiple intradermal injections once in the affected area and the pain reduction lasted for 12 weeks. Large-scale trials and examination of long-term efficacy are needed to test its value for clinical practice.

A phase 2 RCT found that the neuronal nicotinic acetylcholine receptor agonist ABT-594 significantly reduced pain intensity compared with placebo in patients with painful diabetic neuropathy. Unfortunately, ABT-594 treatment was associated with a high number of intolerable dose-related side effects, but the study suggests that further development of this drug class may present new therapeutic options. Intrathecal injections and nerve blocking techniques are other options but relief tends to be temporary.

Stepwise pharmacological approach to the management of neuropathic pain

Figure 13 provides an algorithm for the pharmacological management of Neuropathic pain, and Table 3 provides dosing guidelines for selected agents. Non pharmacological interventions, including physiotherapy, exercise programs and psychological treatment modalities, are also important to improve outcomes. TCAs, gabapentin and pregabalin are all considered first line agents in the management of chronic neuropathic pain. It is reasonable to initiate treatment with either a TCA or an anticonvulsant such as gabapentin or pregabalin. Secondary amine TCAs (nortriptyline and desipramine) are better tolerated than tertiary amine TCAs (amitriptyline and imipramine) and have comparable analgesic efficacy. Amitriptyline, because of its tendency to produce sedation, constipation and urinary retention, should generally be avoided in elderly patients. All antidepressants take approximately two weeks to exert their full analgesic effect at any particular dose, and this needs to be communicated to patients to optimize compliance.

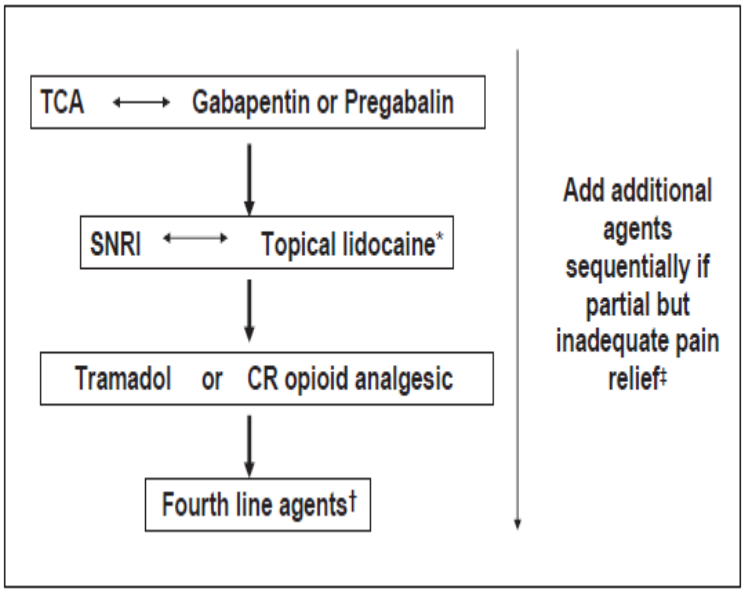

Fig-7 Stepwise pharmacological management of neuropathic pain

Gabapentin and pregabalin appear similar in terms of their mechanisms of action, efficacies and side-effect profiles, and allow for more rapid titration than antidepressant agents. Pregabalin carries the advantage of twice daily dosing and linear pharmokinetics relative to gabapentin. If a TCA fails, switch to an anticonvulsant or vice versa. If a TCA provides only partial pain relief, add an anticonvulsant. The SNRIs are considered to be second line to TCAs because the latter agents provide more robust evidence of efficacy and are much less expensive. However, the TCAs have a more challenging side effect profile and are relatively contraindicated in patients with significant cardiovascular disease.

Topical lidocaine is a good second-line analgesic for an elderly patient with a focal painful neuropathy like post herpetic neuralgia because side effects are usually negligible. When first-line and second-line medications have failed, tramadol or a conventional opioid analgesic may be useful as third-line treatment. It is reasonable to consider a short-acting opioid such as oxycodone with acetaminophen for breakthrough pain during titration of first-line and second-line agents, if needed. If there is an inadequate response, the total daily dose of the short-acting opioid may provide guidance as to the initial maintenance dose of a controlled-release opioid analgesic.

Intractable pain may require treatment with the combination of an antidepressant, an anticonvulsant and an opioid analgesic. Support for combination pharmacotherapy comes

5).Kim HK, Park SK, Zhou JL, Taglialatela G, Chung K,

from a recent study reporting enhanced analgesia with a morphine-gabapentin combination relative to either drug alone.

Although opioid analgesics have a NNT comparable to that of TCAs and perhaps better NNT than anticonvulsants, there are several reasons for their relegation to third-line analgesics for the management of Neuropathic pain. Although tolerance often occurs to sedation, nausea and vomiting (and these latter side effects can be treated with antiemetics), there is very little tolerance to constipation and almost all patients placed on long-term opioid analgesics require a bowel regimen with continued monitoring of bowel function. In addition, periodic monitoring of risk of substance abuse and careful documentation of opioid prescriptions should be undertaken.

Fourth-line agents for the management of Neuropathic pain include cannabinoids, methadone and anticonvulsants with lesser evidence of efficacy such as lamotrigine, topiramate and valproic acid. These should be considered when other options have failed or are not possible. They may be considered adjunctive therapies if there are no concerns regarding polypharmacy or drug interactions.

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