BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2011.04111.x

Alcoholic neuropathy: possible mechanisms and future treatment possibilities

Kanwaljit Chopra & Vinod Tiwari

Pharmacology Research Laboratory, University Institute of Pharmaceutical Sciences, UGC Center of Advanced Study, Panjab University, Chandigarh-160 014, India

Correspondence
Dr Kanwaljit Chopra PhD, MNASc, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India.
Tel.: +91 17 2253 4105
Fax: +91 17 2254 1142
E-mail: dr_chopra_k@yahoo.com

Keywords
acetaldehyde, alcoholic neuropathy, benfotiamine, caspase, protein kinase C, thiamine

Received
24 February 2011

Accepted
19 September 2011

Accepted Article Published Online
11 October 2011

Chronic alcohol consumption produces painful peripheral neuropathy for which there is no reliable successful therapy, mainly due to lack of understanding of its pathobiology. Alcoholic neuropathy involves coasting caused by damage to nerves that results from long term excessive drinking of alcohol and is characterized by spontaneous burning pain, hyperalgesia and allodynia. The mechanism behind alcoholic neuropathy is not well understood, but several explanations have been proposed. These include activation of spinal cord microglia after chronic alcohol consumption, oxidative stress leading to free radical damage to nerves, activation of mGlu5 receptors in the spinal cord and activation of the sympathoadrenal and hypothalamo-pituitary-adrenal (HPA) axis. Nutritional deficiency (especially thiamine deficiency) and/or the direct toxic effect of alcohol or both have also been implicated in alcohol-induced neuropathic pain. Treatment is directed towards halting further damage to the peripheral nerves and restoring their normal functioning. This can be achieved by alcohol abstinence and a nutritionally balanced diet supplemented by all B vitamins. However, in the setting of ongoing alcohol use, vitamin supplementation alone has not been convincingly shown to be sufficient for improvement in most patients. The present review is focused around the multiple pathways involved in the development of peripheral neuropathy associated with chronic alcohol intake and the different therapeutic agents which may find a place in the therapeutic armamentarium for both prevention and management of alcoholic neuropathy.

Introduction

Alcohol is one of the most commonly used substances in the world. After ingestion, alcohol distributes throughout body tissues and rapidly crosses the blood-brain barrier. It is not surprising that ethanol abuse significantly contributes to damage in a variety of tissues including liver, the central and peripheral nervous systems, and skeletal and cardiac muscle. Alcoholic peripheral neuropathy is a potentially incapacitating complication of long-term excessive consumption of alcohol characterized by pain and dysesthesias, primarily in the lower extremities, and is poorly relieved by available therapies [1–3]. Alcohol-related neuropathy is associated with several risk factors, such as malnutrition, thiamine deficiency, direct toxicity of alcohol and a family history of alcoholism [3–6], but it is not clear which of these plays a primary role in inducing neuropathy [7]. In the early stages of alcoholic neuropathy, patients complain of pain in the extremities, which may be severe and has been described as burning or 'like tearing flesh off the bones' and is characterized by spontaneous burning pain, hyperalgesia and allodynia [8].

Prevalence of alcoholic neuropathy

Using the criteria for alcoholism listed in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), studies employing clinical and electrodiagnostic criteria have estimated that in the United States neuropathy is present in 25–66% of defined 'chronic alcoholics'. The factors most directly associated with the development of alcoholic neuropathy include the duration and amount of total lifetime alcohol consumption. Neuropathy is more prevalent in frequent, heavy and continuous drinkers compared with more episodic drinkers [6]. Incidence of alcoholic polyneuropathy was found to be higher in women compared with men [9]. The findings were supported by the results from preclinical studies by Dina *et al.* [10] who also found that alcohol induced neuropathy had

a rapid onset and greater severity in female as compared with male rats.

Clinical symptoms associated with alcoholic peripheral neuropathy

Clinical features of alcoholic peripheral neuropathy develop slowly, extending over a period of months and include abnormalities in sensory, motor, autonomic and gait functions. Painful sensations with or without burning quality represent the initial and major symptom of alcoholic neuropathy [2, 4]. Sometimes, these symptoms can be very painful and incapacitating. Later on, weakness appears in the extremities, involving mainly the distal parts. Progressively, the sensory and motor symptoms and signs extend proximally into the arms and legs and finally the gait may become impaired [11]. Progression of symptoms is usually gradual, continuing over months or years [2, 4]. Electrophysiologic and pathologic findings mainly indicate axonal neuropathy with reduced nerve fibre densities. Densities of small myelinated fibres and unmyelinated fibres were more severely reduced than the density of large myelinated fibres, except in patients with a long history of neuropathic symptoms and marked axonal sprouting [2]. Subperineurial oedema is more prominent in thiamine deficient neuropathy, whereas segmental de/remyelination resulting from widening of consecutive nodes of Ranvier is more frequent in alcoholic neuropathy [3].

Pathophysiology: different pathways involved

The pathogenesis of alcoholic neuropathy is still under debate. It has previously been considered in relationship to nutritional, especially thiamine, deficiencies seen in alcoholics. Thiamine deficiency is closely related to chronic alcoholism and can induce neuropathy in alcoholic patients. Ethanol diminishes thiamine absorption in the intestine, reduces hepatic stores of thiamine and affects the phosphorylation of thiamine, which converts it to its active form [12]. In addition, patients with chronic alcoholism tend to consume smaller amounts of essential nutrients and vitamins and/or exhibit impaired gastrointestinal absorption of these nutrients secondary to the direct effects of alcohol. These relationships make chronic alcoholism a risk factor for thiamine deficiency. In addition to thiamine deficiency, recent studies indicate a direct neurotoxic effect of ethanol or its metabolites. Axonal degeneration has been documented in rats receiving ethanol while maintaining normal thiamine status [5]. Human studies have also suggested a direct toxic effect, since a dose-dependent relationship has been observed between severity of neuropathy and total life time dose of ethanol [6, 13]. The exact mechanism behind alcoholic neuropathy is not well understood, but several explanations have been proposed. These include activation of spinal cord microglia after chronic alcohol consumption [14], activation of mGlu5 receptors in the spinal cord [15], oxidative stress leading to free radical damage to nerves, release of pro-inflammatory cytokines coupled with activation of protein kinase C [16], involvement of extracellular signal-regulated kinases (ERKs) or classical MAP kinases [10], involvement of the opioidergic [14] and hypothalamo-pituitary-adrenal system [17–19]. Some other studies have indicated that chronic alcohol intake can decrease the nociceptive threshold with increased oxidative-nitrosative stress and release of pro-inflammatory cytokines coupled with activation of protein kinase C (Figure 1) [10, 16]. Therefore, alcoholic neuropathy may occur by a combination of the direct toxic effects of ethanol or its metabolites and nutritional deficiencies, including thiamine deficiency. The precise mechanisms responsible for toxicity on the peripheral nervous system, however, have not yet been clarified. The amount of ethanol which causes clinically evident peripheral neuropathy is also still unknown.

Nutritional factors responsible for alcoholic neuropathy (indirect toxicity)

Contribution of metabolic pathways

The primary axonal damage and secondary demyelination of motor and sensory fibres (especially small diameter fibres) are considered to constitute the morphologic basis of alcoholic damage to nerve tissue at present [20]. The demyelination is explained as the result of a slowing down (decceleration) of axoplasmic flow and a degradation of the quality of biological properties of axonal enzymes and proteins. This type of degeneration, so called 'dying-back', resembles Wallerian degeneration. Ethanol and its toxic degradation metabolites affect neuronal metabolism including the metabolic pathways of nucleus, lysosomes, peroxisomes, endoplasmatic reticulum and cytoplasm [21]. Alcohol enters the blood as early as 5 min after ingestion and its absorption peaks after 30–90 min. The key role in the degradation of ethanol is played by ethanol dehydrogenase and acetaldehyde dehydrogenase-two step enzymatic systems by which ethanol is converted to acetate which is further metabolized in humans. Acetaldehyde dehydrogenase is a mitochondrial enzyme which undergoes a single amino acid substitution (mutation) in about 50% of the Asian population in a way similar to the genetic changes in sickle cell anaemia [21]. Thus, in alcoholics with the mutated dehydrogenase enzyme, acetaldehyde concentrations may reach values about 20 times higher than in individuals without the mutation. A certain amount of acetaldehyde is not metabolized by the usual pathways (Figure 2) and binds irreversibly to proteins

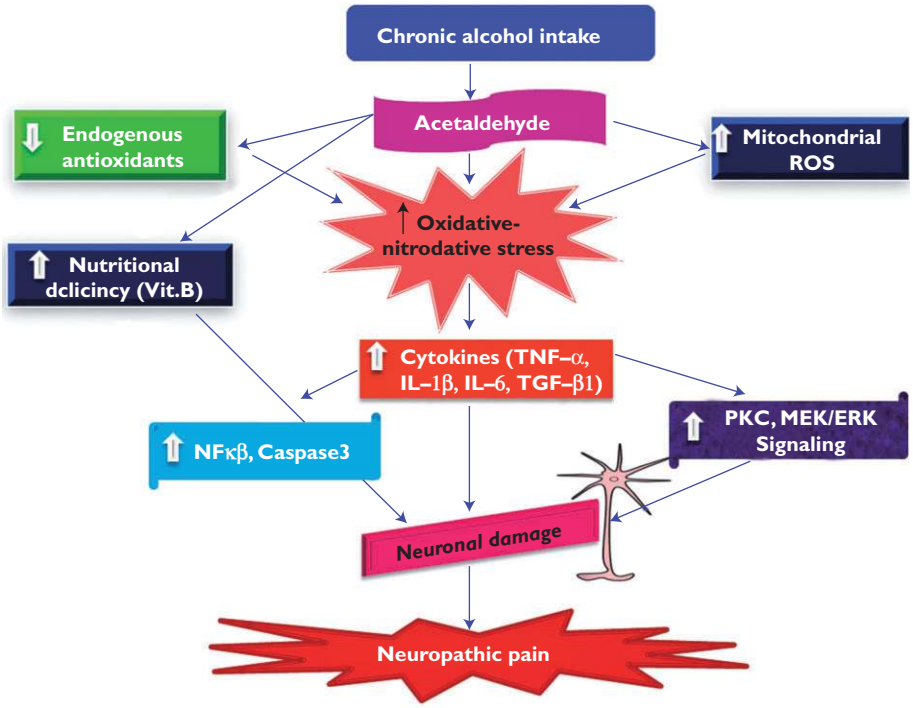

Figure 1

A schematic diagram of different pathways involved in the pathophysiology of alcoholic neuropathy

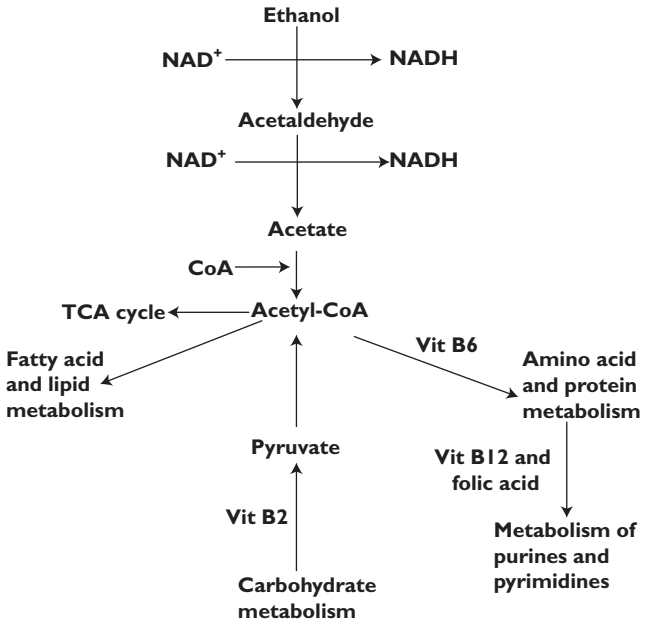

Figure 2

Diagram depicting metabolism of ethanol and its metabolite. CoA coenzyme A, TCA tricarboxylic acid cycle, Vit B6 vitamin B6, Vit B12 vitamin B12

which results in the creation of cytotoxic proteins which adversely affect the function of nervous system cells. These abnormal proteins influence other cell populations especially the hepatocytes where the damage to hepatic mitochondria results in hepatic cirrhosis with reduction of energetic substrates in the liver. The action of these abnormal proteins is explained by competition with normal proteins causing the damage to function and metabolism of the cell [22].

One of the other important issues in alcoholic individuals is the source of their calorie intake. These individuals draw the majority of calories from calorie rich alcoholic beverages with low nutritive value. Chronic abuse of alcohol depletes the pool of liver proteins which are consumed for energy production and insufficient intake of proteins only worsens this imbalance. Resulting disturbances in protein and lipid metabolism lead to undernourishment which adversely influences other metabolic pathways, including those influencing the function of the nervous system. While the central nervous system has its own barrier systems (blood-brain barrier), which may defy the metabolic and toxic influences and their effect on brain functions for a significant period of time, the peripheral nervous system lacks this protective barrier which can contribute to the fact that peripheral nervous system disorders are present in 12–30% of alcohol abusers [23].

Relationship between alcoholic neuropathy and thiamine deficient neuropathy

There is both clinical and experimental evidence of a direct neurotoxic effect of ethanol, while some have argued that it results from a nutritional deficiency, especially thiamine deficiency. The relationships between alcoholic neuropathy and commonly associated nutritional deficiencies, especially thiamine deficiency have been discussed in terms of the apparent clinical and pathologic presentations [24, 25]. Koike *et al*. [26] compared the clinicopathologic features of thiamine-deficiency neuropathy caused by a dietary imbalance with those caused by gastrectomy, including strict biochemical determination of thiamine status. Although clinical manifestations varied widely between patients with either type of thiamine deficiency neuropathy, overall clinicopathologic features, including the spectrum of clinical variability, did not differ significantly by cause. Thus, clinicopathologic features of post gastrectomy polyneuropathy with thiamine deficiency are identical to those of beriberi neuropathy, and the results further confirmed that thiamine deficiency can be a major cause of postgastrectomy polyneuropathy [27]. In another clinical study by Koike *et al*. [28] the cause of the thiamine deficiency was found to be associated with gastrectomy to treat cancer in a 46-year-old man and with dietary imbalance in a 33-year-old man. In both patients, the upper and lower extremities showed a rapidly progressive weakness over the course of 1 month. Muscle weakness in the first patient progressed even after admission to hospital and urinary retention, Wernicke's encephalopathy, lactic acidosis, paralytic ileus and heart failure appeared subsequently. Clinical symptoms in both patients showed improvement after initiation of thiamine administration, although some residual deficit remained. Clinically, sensory disturbance and weakness, especially in the distal part of the lower extremities, are common features of both alcoholic and thiamine deficiency neuropathies [24, 29]. Electrophysiologic and histopathologic findings of axonal neuropathy have also been considered as common features [2, 5, 29, 30]. These similarities have led to a belief that these two neuropathies are identical, and that polyneuropathy associated with chronic alcoholism most likely is caused by thiamine deficiency [24, 25]. Thus, the concept of alcoholic neuropathy encompasses both direct neurotoxicity of ethanol or its metabolites and the concomitant effects of nutritional status, especially thiamine deficiency.

In one clinical study, aimed at studying distinct clinicopathologic features of alcoholic neuropathy, 64 patients were assessed. In 47 of these patients sural nerve biopsy was performed, with discrimination in terms of their thiamine status [3]. The ethanol consumption of these patients was more than 100 g day^{-1} for more than 10 years. These patients were divided into two groups based on thiamine status. The subgroup without thiamine deficiency consisted of 36 patients, while the subgroup with thiamine deficiency consisted of 28 patients. In addition, 32 patients with nonalcoholic thiamine deficiency neuropathy were also evaluated for comparison. The subgroup without thiamine deficiency, considered to be a pure form of alcoholic neuropathy, uniformly showed slowly progressive, sensory dominant symptoms. Superficial sensation, especially nociception, was predominantly impaired and painful symptoms were the primary complaint in most patients in this group. In contrast, the neuropathic symptoms of nonalcoholic thiamine deficiency neuropathy, considered to be identical to beriberi neuropathy [26], were variable, but typically were motor dominant and acutely progressive, affecting both superficial and deep sensation. The histologic features of sural nerve biopsy specimens demonstrated small fibre predominant axonal loss as characteristic of the pure form of alcoholic neuropathy.

Role of nutritional status other than thiamine deficiency

Deficiency of vitamins other than thiamine may also contribute to clinical features of alcoholic neuropathy. Chronic alcoholism can alter the intake, absorption and utilization of various nutrients (nicotinic acid, vitamin B2, vitamin B6, vitamin B12, folate or vitamin E). Deficiencies of B vitamins other than thiamine also may contribute to variation in clinical features, but characteristic symptoms of multiple vitamin deficiency were not seen in patients with thiamine deficiency neuropathies due to gastrectomy and dietary imbalance [26]. These clinical features include anorexia, diarrhoea, erythematous and hyperkeratotic dermatitis, and mental changes in pellagra (nicotinic acid deficiency), cheilosis, glossitis, keratoconjunctivitis and dermatitis in vitamin B2 deficiency and myelopathy in vitamin B12 and folate deficiencies. Thus, these vitamin deficiencies were not considered to be major causal factors of neuropathy [26].

Behse & Buchthal [31] compared 37 Danish patients with alcoholic neuropathy with six patients with nonalcoholic post gastrectomy polyneuropathy. The authors noted that Danish beer at the time of the study contained thiamine and vitamin B6. Thus, deficiency of these vitamins was felt to be unlikely in Danish beer drinkers at that time and, indeed, measured vitamin concentrations were mostly normal. Clinical features of neuropathies in the alcoholic and post gastrectomy patients were similar. These two groups, however, were distinct from the standpoint that nerve conduction velocities were slower and sural nerve biopsy specimens revealed more segmental demyelination in the post gastrectomy group. The authors concluded that malnutrition, including low blood concentrations of B vitamins, is not a prerequisite for the development of alcoholic neuropathy, and ethanol *per se* plays a role in the pathogenesis of alcoholic neuropathy. Another study by Zambelis *et al*. [32] also suggested the participation of the direct toxic effect of ethanol on the peripheral nervous system in the pathogenesis of alcoholic neuropa-

thy, although long standing hyperglycaemia and impaired vitamin B12 utilization were also suggested to be involved.

Direct toxic effects of ethanol or its metabolites (direct toxicity)

Role of acetaldehyde in alcoholic neuropathy

Ethanol can exert its harmful effects through its metabolism. One possible mediator of the direct neurotoxic effect of ethanol is acetaldehyde, a highly toxic metabolite of ethanol with extraordinary reactivity. The mechanisms of the toxicity for liver include production of acetaldehyde-protein adduct formation, depletion of glutathione, microtubular impairment, inhibition of DNA repair, impairment of mitochondrial electron transport chain and stimulation of immunologic reactivity. There is evidence that acetaldehyde-protein adducts are present even in organs that do not seem to produce acetaldehyde efficiently themselves, due to lack of ADH expression [33]. In such cases, acetaldehyde may be formed by induction of the microsomal ethanol oxidizing system [34]. Alternatively, acetaldehyde may reach those organs by blood flow. Given these possibilities, the mechanisms by which acetaldehyde has toxic effects on peripheral nerves may be similar to those in the liver and other organs. Dose-dependent increases in neuronal cell death were demonstrated by incubation of neuronal cell cultures with acetaldehyde-derived advanced glycation end-products (AA-AGE), and the neurotoxicity of AA-AGE is attenuated by the addition of an anti-AAAG-specific antibody [35]. These results suggest that the neurotoxicity due to accumulation of acetaldehyde may be associated with the pathogenesis of alcoholic neuropathy.

Oxidative-nitrosative stress and alcoholic neuropathy

Oxidative stress is known to play a very important role in experimental animal models of neuropathic pain. Lee *et al*. [36] suggested that reactive oxygen species are importantly involved in the development and maintenance of capsaicin-induced pain, particularly in the process of central sensitization in the spinal cord in rats. Padi *et al*. [37], demonstrated that chronic administration of minocycline when started early before peripheral nerve injury could attenuate the development of neuropathic pain by inhibiting pro-inflammatory cytokine release and oxidative and nitrosative stress in mononeuropathic rats. Naik *et al*. [38] suggested the involvement of oxidative stress in experimentally induced chronic constriction injury of the sciatic nerve model in rats. Endoneural oxidative stress leads to nerve dysfunction in rats with chronic constriction injury [39]. A significant decrease in the activity of antioxidant enzymes (superoxide dismutase and catalase) and an increase in lipid peroxidation were observed in sciatic nerves of diabetic rats with established neuropathic pain [40]. ROS triggers second messengers involved in central sensitization of dorsal horn cells [41] or they activate spinal glial cells which in turn play an important role in chronic pain [42]. Reduced glutathione is a major low molecular weight scavenger of free radicals in cytoplasm. Depletion of glutathione increases the susceptibility of neurones to oxidative stress and hyperalgesia [43, 44].

Nitric oxide is also implicated in neuropathic pain [45, 46]. It sensitizes spinal neurones and contributes to sensitization of central neurones by disinhibition [47]. Moreover, unfettered production of nitric oxide coupled with deficient superoxide dismutase leads to production of peroxynitrite, which is several times more potent than its parents in terms of tissue toxicity. Ethanol is oxidized to acetaldehyde by cytochrome P450, which increases reactive oxygen species, with concomitant changes in redox balance [48, 49]. Rats given chronic ethanol show enhanced production of oxidative markers, such as thiobarbituric acid reactive substances, hydrogen peroxide and OH^- like species [50]. Studies have suggested that chronic ethanol increases oxidative damage to proteins, lipids and DNA [51, 52]. Bosch-Morell *et al*. [53] demonstrated that chronic ethanol promotes oxidative stress in rat peripheral nerve. The amount of malondialdehyde, a lipid peroxidation product, increases in the sciatic nerves of rats fed an ethanol-containing diet, when compared with pair-fed animals. Moreover, glutathione content and glutathione peroxidase activity in this same tissue decrease in ethanol-fed *vs*. pair-fed rats, suggesting the probable involvement of alcohol induced oxidative stress in the pain like state associated with chronic alcohol intake. Recently, we have also found a significant increase in lipid peroxide concentrations and marked decrease in reduced glutathione, superoxide dismutase and catalase activity in the sciatic nerve of rats with hyperalgesia and allodynia given alcohol (10 g kg^{-1} orally) for 10 weeks [54, 55]. Thus, following ethanol intoxication, the balance between pro-oxidants and anti-oxidants is disturbed to such an extent that it results in the oxidative damage of biomolecules, such as fats, proteins or DNA, finally leading to cell injury and thus alcoholic neuropathy.

Molecular mechanisms involved in alcoholic neuropathy

Role of protein kinases in alcoholic neuropathy

Protein kinase C (PKC) is a family of protein kinases consisting of approximately 10 isozymes. PKC is involved in receptor desensitization, modulating membrane structure events, regulating transcription, mediating immune responses, regulating cell growth and in learning and memory. These functions are achieved by PKC mediated phosphorylation of other proteins [16]. Apart from above function, over-activation of epsilon form of protein kinase C (PKCε) is known to be involved in mediating neuropathic

pain, such as pain induced by cancer chemotherapy (vincristine) [56] and diabetes [57]. PKC and protein kinase A (PKA) are both known to be important in nociceptor function [57–59]. There are several studies suggesting the involvement of protein kinases in alcoholic neuropathy. Dina *et al*. [16] maintained rats on a diet to simulate chronic alcohol consumption in humans and found mechanical hyperalgesia by the fourth week which was maximal at 10 weeks. Thermal hyperalgesia and mechanical allodynia were also present with decreased mechanical threshold of C-fibres. The hyperalgesia was acutely attenuated by intradermal injection of nonselective PKC or selective PKCε inhibitors injected at the site of nociceptive testing. Western immunoblot analysis indicated a higher level of PKCε in dorsal root ganglia from alcohol-fed rats, supporting a role for enhanced PKCε second messenger signalling in nociceptors contributing to alcohol-induced hyperalgesia [16]. Miyoshi *et al*. [15] found that a significant decrease in the mechanical nociceptive threshold was observed after 5 weeks of chronic ethanol consumption in rats. This hyperalgesia was significantly attenuated by repeated i.p. injection of (S)-2,6-diamino-N-[[1-(oxotridecyl)-2-piperidinyl] methyl] hexanamide dihydrochloride (NPC15437), a selective PKC inhibitor, once a day for a week after 4 weeks of ethanol treatment. Moreover, phosphorylated PKC was significantly increased in the spinal cord following chronic ethanol consumption. These findings constitute direct evidence that spinal PKC plays a substantial role in the development and maintenance of an ethanol-dependent neuropathic pain-like state in rats.

PKA and PKCε signalling is also known to play a highly sexually dimorphic role in alcoholic neuropathy [10]. In gonad-intact female rats, Walsh inhibitor peptide (WIPTIDE), both a PKCε inhibitor as well as a PKA inhibitor, injected intradermally at the site of nociceptive testing after establishing alcohol induced hyperalgesia, significantly inhibited hyperalgesia. Following ovariectomy, alcohol failed to induce hyperalgesia in female rats while oestrogen replacement reinstated alcoholic neuropathy in the female rats. The PKA inhibitor, WIPTIDE, also attenuated alcohol-induced hyperalgesia in oestrogen-replaced female rats. In addition, the magnitude of analgesia induced by a PKCε inhibitor was greater in female as compared with male rats. However, in male rats, a PKCε inhibitor, but not a PKA inhibitor, attenuated alcohol-induced hyperalgesia [10]. The mechanism underlying the sexually dimorphic contribution of PKA and PKCε to pain associated with alcohol-induced neuropathy remains to be determined.

A connection between MEK/ERK signaling and alcoholic neuropathy

Extracellular signal-regulated kinases (ERKs) or classical mitogen activated protein (MAP) kinases (also known as MEK) are widely expressed protein kinase intracellular signalling molecules which are involved in functions including the regulation of meiosis, mitosis and post mitotic functions in differentiated cells. Many different stimuli, including growth factors, cytokines, viral infection, ligands for heterotrimeric G protein-coupled receptors, transforming agents, and carcinogens, activate the ERK pathway. There are many studies suggesting the role of MEK/ERK signaling in inflammatory pain in male [60–63] and female rats [64]. Dina *et al*. [10] evaluated the contribution of MEK/ERK to alcohol-induced peripheral neuropathy and found that intradermal injection of PD98059 (1 μg μl^{-1}), a selective inhibitor of mitogen and ERK kinase and U0126 (1 μg μl^{-1}), a specific inhibitor of ERK1/2, after establishing a state of hyperalgesia in alcohol-fed rats of either gender, attenuated ethanol induced hyperalgesia similarly in male and female rats, consistent with a comparable role for MEK/ERK signaling in chronic alcohol-induced hyperalgesia in rats of both genders.

Role of spinal cord microglia

Spinal cord glial cells are implicated in the exaggerated pain state created by diverse manipulations such as subcutaneous inflammation, neuropathy and spinal immune activation [65, 66]. It has been recognized that spinal cord glial cells, astrocytes and microglia are activated by neuropathic pain or peripheral inflammation [42]. Furthermore, astrocytes and microglia are activated by such pain relevant substances as substance P, calcitonin-gene related peptide (CGRP), ATP and excitatory amino acids from primary afferent terminals, in addition to viruses and bacteria [67, 68]. In a study by Narita *et al*. [14], 5 weeks ethanol treatment resulted in significantly decreased mechanical nociceptive threshold along with microglia activation in the spinal cord of rats, implicating a role for proliferated and activated microglia in the expression of a neuropathic pain-like state following chronic ethanol consumption.

Role of caspases in alcoholic neuropathy

Caspases, or cysteine-aspartic acid proteases, are a family of cysteine proteases, which play an essential role in apoptosis (programmed cell death), necrosis and inflammation. Translocation of NFkβ to the nucleus has been reported to result in activation of the endogenous proteolytic enzyme system caspases [69]. Consequently, the cascade events promote further apoptosis [70]. Joseph & Levine [71] suggested that activity in signaling pathways that ultimately lead to apoptosis plays a critical role in the generation of neuropathic pain, before death of sensory neurones becomes apparent. Activator and effector caspases, defining components of programmed cell death signalling pathways, also contribute to pain-related behaviour in animals with small fibre peripheral neuropathies. The death receptor ligand, tumour necrosis factor α, and its downstream second messenger, ceramide, also produce pain-related behaviour via this mechanism. In two models of painful peripheral neuropathy, HIV/AIDS therapy (induced by the nucleoside reverse transcriptase inhibitor,

dideoxycytidine) and cancer chemotherapy (induced by vincristine) peripheral neuropathy, and for pain-related behaviour induced by tumour necrosis factor α and its second messenger, ceramide, inhibition of both activator (1, 2, 8 and 9) and effector (3) caspases attenuates neuropathic pain-related behaviour. This suggests that these pathways are potential targets for novel pharmacological agents for the treatment of inflammatory as well as neuropathic pain [71].

Chronic exposure to ethanol results in increased amounts of oxidative damage; translocation of PKC, activation of PKC and NFκβ, which results in DNA fragmentation and ultimately increased neuronal death through apoptosis or other mechanisms that are responsible for the behavioural deficits [72]. Izumi *et al*. [73] also demonstrated that a single day of ethanol exposure in rats on post natal day 7 results in significant apoptotic neuronal damage throughout the forebrain after 24 h of ethanol administration. Thus, it is quite possible that chronic alcohol consumption is responsible for inducing neuropathy by activation of the caspase cascade and may be an important target for the treatment of alcoholic neuropathy.

Involvement of glutamate receptors

Accumulating evidence suggests a pivotal role for metabotropic glutamate receptors (mGluRs) in nociceptive processing, inflammatory pain and hyperalgesia [74, 75]. Several mGluR subtypes have been identified in the superficial dorsal horn of the spinal cord [76, 77] and on primary afferent fibres [78]. Glutamate concentrations are elevated in the superficial dorsal horn of rats after chronic ligature of the sciatic nerve [79]. Miyoshi *et al*. found that 5 weeks after ethanol treatment, the mechanical nociceptive threshold was significantly decreased and is further reduced up to 10 weeks [80]. As supported by immunostaining, the membrane fraction showed that spinal mGluR5 concentrations in ethanol-treated rats were significantly increased compared with those in the control diet group. These findings support the idea that the increased number of membrane-bound mGluR5 following chronic ethanol consumption may lead to a long lasting activation of neuronal protein kinase C in the dorsal horn of the spinal cord. This phenomenon may be responsible for the induction of the neuropathic pain like behaviour following chronic ethanol consumption. Not only mGluRs but ionotropic glutamate (NMDA) receptors are also involved in alcoholic-induced neuropathic pain. Narita *et al*. [14] found that the p- Ser1303-NR2B subunit protein (subunit of NMDA receptor) in the spinal cord of rats was significantly increased following chronic ethanol treatment suggesting that PKC-dependent NR2BRs in the spinal cord may be activated following chronic ethanol consumption and may be involved in the induction of the ethanol dependent neuropathic pain-like state.

Involvement of the opioidergic system

Narita *et al*. [14] found that chronic alcohol consumption was associated with long lasting hyperalgesia during and even after ethanol withdrawal along with opioid receptor dysfunctioning specific for μ opioid receptors (MOR), but not delta and kappa opioid receptors. These findings suggest that chronic ethanol treatment causes the specific dysfunction of MOR. Thus, up-regulation of cPKC activity may, at least in part, be involved in MOR dysfunction (may be an increase in MOR phosphorylation) following chronic ethanol treatment. This phenomenon may explain the reduced sensitivity to morphine-induced antinociception under the ethanol-dependent neuropathic pain-like state.

Involvement of the sympatho-adrenal and hypothalamo-pituitary-adrenal (HPA) axis in alcoholic peripheral neuropathy

Alcohol consumption potently activates the two major neuroendocrine stress axes, leading to the sustained release of glucocorticoids and catecholamines [17–19]. Increased activity in the sympathetic nervous system has been implicated in some forms of neuropathic pain [81, 82] and glucocorticoids have been reported to exacerbate pain in some animal models of peripheral neuropathy [83]. Dina *et al*. demonstrated the involvement of the sympatho-adrenal stress axis and its final common mediator, epinephrine, in painful alcoholic neuropathy by showing that adrenal medullectomy prevented and reversed the pro-nociceptive effects of alcohol consumption [84]. Moreover, they found that the hyperalgesic phenotype in rats which had undergone adrenal medullectomy by administering stress levels of epinephrine was reconstituted. The critical contribution of stress hormones to the pain associated with alcohol-induced peripheral neuropathy, combined with the demonstration of stress-induced hyperalgesia, dependent on neuroendocrine stress axes [85, 86], suggests that the mechanisms described in the study of Dina *et al*. may have implications for other types of pain in which patients experience repeated exposure to stress [84].

Thus, from the above discussion it is clear that stress hormones, catecholamines and glucocorticoids, from the sympatho-adrenal and HPA neuroendocrine stress axes, respectively, play a very important role in initation and maintenance of alcoholic neuropathy. The combined actions of catecholamines and glucocorticoids, via their receptors on sensory neurones, demonstrate a novel mechanism by which painful alcoholic neuropathy is induced and maintained.

Effects on axonal transport and cytoskeletal properties

Axonal transport and cytoskeletal properties are impaired by ethanol exposure [4]. Since alcoholic neuropathy

manifests with length-dependent axonal degeneration, the axonal transport system, which supplies essential proteins and other cellular components, may be the primary site exhibiting vulnerability to the toxicity of ethanol. Yerdelen *et al*. suggest that alcoholic neuropathy is a primary axonal neuropathy characterized by Wallerian degeneration of the axons and a reduction in the myelination of neural fibres [87]. An *in vitro* study of axonal transport using dorsal root ganglion-sciatic nerve preparations from the rat showed that transport was reduced following long term ethanol feeding [88]. *In vivo* studies using rats have demonstrated impairment of retrograde axonal transport [89, 90]. Ethanol exposure reduces neurofilament protein concentrations in primary cultured hippocampal neurones [91]. Studies using the rat spinal cord indicate that chronic ethanol exposure causes a reduction in neurofilament-associated phosphatase activity and an increase in phosphate content of neurofilament proteins [92]. An *in vitro* study using rat brain has demonstrated that phosphorylation of microtubule-associated proteins, which modulate the functional properties of microtubules, is altered by ethanol exposure [93]. A study using hepatoma-derived cells has shown altered integrity of proteins associated with microtubules following ethanol exposure [94]. Altered expression of neuronal protein 22, which interacts with microfilament and microtubule matrices, may also be involved in the pathogenesis of alcoholic neuropathy [95]. Thus, defects in axonal transport and cytoskeletal properties of axons may be one of the important pathways involved in alcohol induced peripheral neuropathy.

Thus, it is clear that all the above pathways are potential targets for novel pharmacological agents for the treatment of alcoholic neuropathy.

Clinical management of alcoholic neuropathy

Treatment is directed towards halting further damage to the peripheral nerves and returning to normal functioning. This can be achieved by alcohol abstinence, a nutritionally balanced diet supplemented by all B vitamins, and rehabilitation. However, in the setting of ongoing ethanol use, vitamin supplementation alone has not been convincingly shown to be sufficient for improvement in most patients. Painful dysesthesias associated with alcoholic neuropathy can be treated using gabapentin or amitriptyline with other over the counter pain medications, such as aspirin or acetaminophen. However these drugs are being used only for the management of acute pain and are ineffective in targeting the basic pathological pathways involved in alcoholic neuropathy.

Here we discuss a few of the therapeutic options which are tried and could be tried for prevention and treatment of alcoholic peripheral neuropathy.

Benfotiamine for the treatment of alcohol related peripheral neuropathy

Benfotiamine (*S*-benzoylthiamine O-monophoshate) is a synthetic S-acyl derivative of thiamine (vitamin B1). A deficiency of vitamin B1 in chronic alcoholics can be due to inadequate dietary intake, reduced capacity for hepatic storage, inhibition of intestinal transport and absorption or decreased formation of the active coenzyme form. In an animal study, it has been found that chronic alcohol consumption in rats resulted in a significant depletion in thiamine diphosphate (TDP), the active coenzyme form of thiamine. Supplementation with benfotiamine significantly increased concentrations of TDP and total thiamine compared with supplementation with thiamine HCl [96]. An 8 week, randomized, multicentre, placebo-controlled, double-blind study compared the effect of benfotiamine alone with a benfotiamine complex (Milgamma-N) or placebo in 84 alcoholic patients. Parameters measured included vibration perception in the great toe, ankle and tibia, neural pain intensity, motor function and paralysis, sensory function and overall neuropathy score and clinical assessment. Although benfotiamine therapy was superior to Milgamma-N or placebo for all parameters, results reached statistical significance only for motor function, paralysis and overall neuropathy score. The reason for better results in the benfotiamine alone group than in the Milgamma-N group, despite the fact that the benfotiamine dosage was equivalent, is not completely understood. The authors hypothesized that vitamins B6 and B12 might have competed with the effects of vitamin B1 in the Milgamma-N group [97]. In another small Russian study, 14 chronic alcoholic men with polyneuropathy were given 450 mg benfotiamine daily for 2 weeks, followed by 300 mg daily for an additional 4 weeks. During the treatment the regression of neuropathy symptoms, other sensor and movement disorders were observed. The evidence of positive dynamics at peripheral and segmental nerve system level was supported by neurophysiological data. Benfotiamine was found to be beneficial in patients with alcoholic polyneuropathy [98].

Alpha-lipoic acid

Alpha-lipoic acid, the most well-researched nutrient for peripheral neuropathy, has been used as a treatment for peripheral neuropathy in Europe for decades. Several studies examining the mechanism of alpha-lipoic acid have been conducted on streptozotocin-diabetic rats with neuropathy. Alpha-lipoic acid was found to increase glucose uptake by nerve cells [99], nerve myo-inositol [99, 100], glutathione concentrations [100, 101], (Na^+/K^+)-ATPase activity, nerve blood flow and normalize NAD : NADH ratios [100].

Thus, alpha-lipoic acid may have a potential in the treatment of patients with alcoholic neuropathy.

Acetyl-L-carnitine

Acetyl-L-carnitine has been tested in clinical [102] and animal studies [103] for the treatment of chemotherapy-induced peripheral neuropathy. The decreases in nerve conduction velocity were significantly less in groups supplemented with acetyl-L-carnitine. In addition, acetyl-L-carnitine did not interfere with the antitumour effects of the drugs.

Thus, there is a need to screen acetyl-L-carnitine in both preclinical and clinical models of alcoholic neuropathy.

Vitamin E

Vitamin E is used to refer to a group of fat-soluble compounds that include both tocopherols and tocotrienols. Treatment with vitamin E was found to be beneficial in the treatment of patients with diabetic peripheral neuropathy [104] and neuropathic pain in streptozotocin-induced diabetic rats [105]. Recently findings from our laboratory also suggest the benefecial effects of both α-tocopherol and tocotrienol, isoforms of vitamin E, in the prevention of hyperalgesia and allodynia in rats administered ethanol for 10 weeks [55]. We found more potent effects with tocotrienol as compared with α-tocopherol [55].

Thus, there is an urgent need to screen the vitamin E isoforms, especially tocotrienol for evaluating clinical efficacy in patients with alcoholic neuropathy.

Methylcobalamin for the treatment of peripheral neuropathy

Vitamin B12 deficiency has been associated with significant neurological pathology, including peripheral neuropathy. Testing serum metabolites such as methylmalonic acid and homocysteine can help identify clinically individuals at risk for a deficiency-associated neurological syndrome [106]. One of the mechanisms believed to be at play in vitamin B12 deficiency neuropathy is hypomethylation in the central nervous system. Inhibition of the B12- dependent enzyme methionine synthase results in a fall in the ratio of S-adenosylmethionine (SAM) to S-adenosylhomocysteine [107] and the resultant deficiency in SAM impairs methylation reactions in the myelin sheath. Clinical trials of methylcobalamin alone or vitamin B12 combined with other B vitamins found overall symptomatic relief of neuropathy symptoms was more pronounced than electrophysiological findings [108]. Hence, future studies are required to test the efficacy of methylcobalamin in both the preclinical and clinical domain.

Myo-inositol for treatment of peripheral neuropathy

Myo-inositol is an important constituent of the phospholipids that make up nerve cell membranes. Because low nerve myo-inositol concentrations have been observed in the pathogenesis of diabetic neuropathy, the potential for supplementation has been explored. Sural nerve biopsies were conducted on 30 male subjects, 10 with type 1 diabetes (five with clinical signs of diabetic neuropathy), 10 with impaired glucose tolerance and 10 with normal glucose tolerance. Nerve myo-inositol concentrations were significantly lower in diabetics with neuropathy. Also, in subjects with normal or impaired glucose tolerance, high nerve myo-inositol concentrations were associated with nerve regeneration as illustrated by increased nerve fibre density [109]. In an animal model of experimental diabetic neuropathy a significant decrease in motor nerve conduction velocity was observed. Supplementation with 500 mg myo-inositol/rat/day partially prevented this decrease, while supplementation with an analogue of myo-inositol, D-myo-inositol-1,2,6-trisphosphate, at a dose of 24 mg/rat/day completely prevented a reduction in nerve conduction velocity [110].

Thus, further studies are required to find whether treatment with myo-inositol can treat symptoms associated with alcoholic neuropathy as the disease pathology also involves nerve fibre degeneration and loss.

The application of N-acetylcysteine for peripheral neuropathy

N-acetylcysteine, an amino acid, is a potent antioxidant and helps to enhance glutathione concentrations. N-acetylcysteine may have application in the prevention or treatment of neuropathy. Rats with experimentally-induced diabetes for 2 months had a 20% reduction in nerve conduction velocity and 48% reduction in endoneurial blood flow. Both were largely corrected by N-acetylcysteine supplementation [111]. A mechanism of cisplatin chemotherapy-induced peripheral neuropathy was elucidated in an *in vitro* mouse model. Apoptosis of neurones was induced by cisplatin, but pre-incubation with N-acetylcysteine completely blocked apoptosis [112].

Thus, further preclinical and clinical studies are required to assess of this molecule in alcoholic neuropathy.

Topical capsaicin cream for the treatment of peripheral neuropathy

Capsaicin is an active principal of the herb *Capsicum officinalis* and is believed to stimulate afferent C-fibres (fibres in the mechano-heat class). The initial stimulation of C-fibres results in burning and irritation that stimulates release of substance P and other neuropeptides. Repeated exposures result in a diminution of the initial burning and irritation and a long-lasting analgesic effect [113]. In a large, multicentre, double-blind, placebo controlled trial conducted by The Capsaicin Study Group, 277 subjects entered the study, 252 continued for at least 2 weeks and 219 completed the 8 week trial. Subjects applied 0.075% capsaicin cream (n = 100 completers) or placebo cream (n = 119 completers) four times daily and were evaluated at 2 week intervals for 8 weeks. Pain was assessed via physician assessment as well as a patient driven visual analogue scale. Statistically significant improvements were noted in physician global

assessment (69.5% *vs.* 53.4%), pain intensity (38.1% *vs.* 27.4%) and degree of pain relief (58.4% *vs.* 45.3%) in the capsaicin *vs.* placebo groups, respectively [114].

Therefore, topical application with capsaicin may provide symptomatic relief from neuropathic pain in patients suffering from alcoholic neuropathy.

Antidepressants for the alleviation of neuropathic pain symptoms

Tricyclic antidepressants (TCAs) are often the first line drugs to alleviate neuropathic pain symptoms. They have central effects on pain transmission and block the active re-uptake of norepinephrine and serotonin. TCAs have been shown to relieve various neuropathic pain conditions in many trials [115]. In agreement with this, one recent study has confirmed the efficacy of TCAs in central pain [116]. The serotonin/norepinephrine re-uptake inhibitors (SNRIs), duloxetine and venlafaxine, have a well-documented efficacy in painful polyneuropathy [117, 118]. SSRIs have been studied in a few trials which have demonstrated a weak analgesic effect but the clinical relevance of these compounds is questionable [119].

Thus, treatment with TCAs may provide symptomatic relief in patients with alcoholic neuropathy.

Anticonvulsants

Antiepileptic drugs, such as the gamma aminobutyric acid (GABA) analogue (gabapentin), have proven helpful in some cases of neuropathic pain. These drugs have central and peripheral anticholinergic effects, as well as sedative effects, and they block the active re-uptake of norepinephrine and serotonin. Recently, extended release gabapentin relieved symptoms of painful polyneuropathy [120]. Lamotrigine was effective in relieving central post stroke pain [121] and painful diabetic polyneuropathy [122], but recent larger studies have failed to show a pain relieving effect in mixed neuropathic pain [123] and painful polyneuropathy [124]. Valproate demonstrated varying effects in different studies of neuropathic pain, with three studies from one group reporting high efficacy [125–127] and others failing to find an effect [128, 129]. Lacosamide, a new anticonvulsant drug, had a small but significant pain relieving effect on painful diabetic neuropathy [130], while subsequent trials have failed to find an effect, except for the efficacy of a 400 mg dose in subgroup analyses [131, 132].

Thus, treatment with anticonvulsant drugs may provide another therapeutic alternative for the symptomatic relief of pain in patients with alcoholic neuropathy.

Conclusion and future perspective

Alcoholic peripheral neuropathy presents with considerable morbidity and can result in significant decreases in quality of life. While conventional medicine can offer some relief, the potential side effects or addictive nature of many of the medications render long term use undesirable. Such treatments, furthermore, merely mask the symptoms and do not address the underlying pathologies. Alternative therapies, on the other hand, are typically without side effects and address nutrient deficiencies, oxidative stress and other aetiological factors associated with the development of peripheral neuropathy.

Benfotiamine, alpha-lipoic acid, acetyl-L-carnitine and methylcobalamin are among the well-researched alternative options for the treatment of peripheral neuropathy. Other potential nutrient or botanical therapies include vitamin E, myo-inositol, N-acetylcysteine and topical capsaicin. Thus there is a need to investigate all the above mentioned agents in animal models of alcoholic neuropathy as well clinically in patients suffering from this disorder. The use of well-researched nutrients and the possible addition of new cutting-edge treatments should decrease the morbidity associated with peripheral neuropathy and the side effects associated with the commonly prescribed conventional pain-relieving treatments in current favour.

As yet there is no effective therapeutic intervention available for relieving the neuropathic pain due to chronic alcohol consumption. Thus there is a need to understand the basic pathophysiological mechanisms involved in alcohol induced neuropathic pain so that new therapeutic modalities targeting disrupted molecular events can be developed for prevention as well as clinical management of alcoholic neuropathy.

Competing Interests

There are no competing interests to declare.

REFERENCES

1 Ratcliff EV. Alcoholic neuropathies. Aust Fam Physician 1979; 8: 171–7.

2 Koike H, Mori K, Misu K, Hattori N, Ito H, Hirayama M, Sobue G. Painful alcoholic polyneuropathy with predominant small-fiber loss and normal thiamine status. Neurology 2001a; 56: 1727–32.

3 Koike H, Iijima M, Sugiura M, Mori K, Hattori N, Ito H, Hirayama M, Sobue G. Alcoholic neuropathy is clinicopathologically distinct from thiamine-deficiency neuropathy. Ann Neurol 2003; 54: 19–29.

4 Koike H, Sobue G. Alcoholic neuropathy. Curr Opin Neurol 2006; 19: 481–6.

5 Bosch EP, Pelham RW, Rasool CG, Chatterjee A, Lash RW, Brown L, Munsat TL, Bradley WG. Animal models of alcoholic neuropathy: morphologic, electrophysiologic, and biochemical findings. Muscle Nerve 1979; 2: 134–44.

6 Monforte R, Estruch R, Valls-Solé J, Nicolás J, Villalta J, Urbano-Marquez A. Autonomic and peripheral

neuropathies in patients with chronic alcoholism. A dose-related toxic effect of alcohol. Arch Neurol 1995; 52: 45–51.

7 Palliyath S, Schwartz BD. Peripheral nerve functions improve in chronic alcoholic patients on abstinence. J Stud Alcohol 1993; 54: 684–6.

8 Brain L, Walton JN. Disorders of peripheral nerves: alcoholic polyneuritis. In: Brain's Diseases of the Nervous System. London: Oxford University Press, 1969; 817–19.

9 Ammendola A, Gemini D, Iannaccone S, Argenzio F, Ciccone G, Ammendola E, Serio L, Ugolini G, Bravaccio F. Gender and peripheral neuropathy in chronic alcoholism: a clinical-electroneurographic study. Alcohol Alcohol 2000; 35: 368–71.

10 Dina OA, Gear RW, Messing RO, Levine JD. Severity of alcohol-induced painful peripheral neuropathy in female rats: role of estrogen and protein kinase (A and C epsilon). Neuroscience 2007; 145: 350–6.

11 Hawley RJ, Kurtzke JF, Armbrustmacher VW, Saini N, Manz H. The course of alcoholic-nutritional peripheral neuropathy. Acta Neurol Scand 1982; 66: 582–89.

12 Singleton CK, Martin PR. Molecular mechanisms of thiamine utilization. Curr Mol Med 2001; 1: 197–07.

13 Ammendola A, Tata MR, Aurilio C, Ciccone G, Gemini D, Ammendola E, Ugolini G, Argenzio F. Peripheral neuropathy in chronic alcoholism: a retrospective cross-sectional study in 76 subjects. Alcohol Alcohol 2001; 36: 271–75.

14 Narita M, Miyoshi K, Narita M, Suzuki T. Involvement of microglia in the ethanol-induced neuropathic pain-like state in the rat. Neurosci Lett 2007; 414: 21–5.

15 Miyoshi K, Narita M, Takatsu M, Suzuki T. mGlu5 receptor and protein kinase C implicated in the development and induction of neuropathic pain following chronic ethanol consumption. Eur J Pharmacol 2007; 562: 208–11.

16 Dina OA, Barletta J, Chen X, Mutero A, Martin A, Messing RO, Levine JD. Key role for the epsilon isoform of protein kinase C in painful alcoholic neuropathy in the rat. J Neurosci 2000; 20: 8614–9.

17 Gianoulakis C, Dai X, Brown T. Effect of chronic alcohol consumption on the activity of the hypothalamic-pituitary-adrenal axis and pituitary beta-endorphin as a function of alcohol intake, age, and gender. Alcohol Clin Exp Res 2003; 27: 410–23.

18 Thayer JF, Hall M, Sollers JJ 3rd, Fischer JE. Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: evidence for impaired inhibitory control of the HPA axis in heavy drinkers. Int J Psychophysiol 2006; 59: 244–50.

19 Walter M, Gerhard U, Gerlach M, Weijers HG, Boening J, Wiesbeck GA. Cortisol concentrations, stress-coping styles after withdrawal and long-term abstinence in alcohol dependence. Addict Biol 2006; 11: 157–62.

20 Ludin HP, Tackman W. Polyneuropathien. Stuttgart, New York: Georg Thieme Verlag, 1984; 250–55.

21 Kucera P, Balaz M, Varsik P, Kurca E. Pathogenesis of alcoholic neuropathy. Bratisl Lek Listy 2002; 103: 26–9.

22 Achord JL. Alcohol and the liver. Sci Amer Sci Med 1995; 2: 16–25.

23 Scheid W. Lehrbuch der Neurologie. Vierte Auflage. Stuttgart: Georg Thieme Verlag, 1980.

24 Victor M, Adams RD. On the etiology of the alcoholic neurologic diseases with special reference to the role of nutrition. Am J Clin Nutr 1961; 9: 379–97.

25 Novak DJ, Victor M. The vagus and sympathetic nerves in alcoholic polyneuropathy. Arch Neurol 1974; 30: 273–84.

26 Koike H, Iijima M, Mori K, Hattori N, Ito H, Hirayama M, Sobue G. Postgastrectomy polyneuropathy with thiamine deficiency is identical to beriberi neuropathy. Nutrition 2004; 20: 961–6.

27 Koike H, Misu K, Hattori N, Ito S, Ichimura M, Ito H, Hirayama M, Nagamatsu M, Sasaki I, Sobue G. Postgastrectomy polyneuropathy with thiamine deficiency. J Neurol Neurosurg Psychiatry 2001b; 71: 357–62.

28 Koike H, Ito S, Morozumi S, Kawagashira Y, Iijima M, Hattori N, Tanaka F, Sobue G. Rapidly developing weakness mimicking Guillain-Barré syndrome in beriberi neuropathy: two case reports. Nutrition 2008; 24: 776–80.

29 Ohnishi A, Tsuji S, Igisu H, Murai Y, Goto I, Kuroiwa Y, Tsujihata M, Takamori M. Beriberi neuropathy: morphometric study of sural nerve. J Neurol Sci 1980; 45: 177–90.

30 Tackmann W, Minkenberg R, Strenge H. Correlation of electrophysiological and quantitative histological findings in the sural nerve of man. Studies on alcoholic neuropathy. J Neurol 1977; 216: 289–99.

31 Behse F, Buchthal F. Alcohol neuropathy: clinical, electrophysiological and biopsy findings. Ann Neurol 1977; 2: 95–110.

32 Zambelis T, Karandreas N, Tzavellas E, Kokotis P, Liappas J. Large and small fiber neuropathy in chronic alcohol-dependent subjects. J Peripher Nerv Syst 2005; 10: 375–81.

33 Masaki T, Mochizuki H, Matsushita S, Yokoyama A, Kamakura K, Higuchi S. Association of aldehyde dehydrogenase-2 polymorphism with alcoholic polyneuropathy in humans. Neurosci Lett 2004; 363: 288–90.

34 Lieber CS. Hepatic and other medical disorders of alcoholism: from pathogenesis to treatment. J Stud Alcohol 1998; 59: 9–25.

35 Takeuchi M, Saito T. Cytotoxicity of acetaldehyde-derived advanced glycation end-products (AA-AGE) in alcoholic-induced neuronal degeneration. Alcohol Clin Exp Res 2005; 29: (Suppl.): 220S–4S.

36 Lee I, Kim HK, Kim JH, Chung K, Chung JM. The role of reactive oxygen species in capsaicin-induced mechanical hyperalgesia and in the activities of dorsal horn neurons. Pain 2007; 133: 9–17.

37 Padi SS, Kulkarni SK. Minocycline prevents the development of neuropathic pain, but not acute pain: possible anti-inflammatory and antioxidant mechanisms. Eur J Pharmacol 2008; 601: 79–87.

38 Naik AK, Tandan SK, Dudhgaonkar SP, Jadhav SH, Kataria M, Prakash VR, Kumar D. Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-l-cysteine in rats. Eur J Pain 2006; 10: 573–9.

39 Kim HK, Park SK, Zhou JL, Taglialatela G, Chung K, Coggeshall RE, Chung JM. Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain. Pain 2004; 111: 116–24.

40 Sharma SS, Sayyed SG. Effects of Trolox on nerve dysfunction, thermal hyperalgesia and oxidative stress in experimental diabetic neuropathy. Clin Exp Pharmacol Physiol 2006; 33: 1022–102.

41 Ali DW, Salter MW. NMDA receptor regulation by Src kinase signalling in excitatory synaptic transmission and plasticity. Curr Opin Neurobiol 2001; 11: 336–42.

42 Raghavendra V, Tanga FY, DeLeo JA. Inhibition of microglia activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther 2003; 306: 624–30.

43 Cooper AJ, Kristal BS. Multiple roles of glutathione in the central nervous system. Biol Chem 1997; 378: 793–802.

44 Wullner U, Seyfried J, Groscurth P, Beinroth S, Winter S, Gleichmann M, Heneka M, Löschmann P, Schulz JB, Weller M, Klockgether T. Glutathione depletion and neuronal cell death: the role of reactive oxygen intermediates and mitochondrial function. Brain Res 1999; 826: 53–62.

45 Levy D, Zochodne DW. NO pain: potential roles of nitric oxide in neuropathic pain. Pain Pract 2004; 4: 11–8.

46 Sung CS, Wen ZH, Chang WK, Ho ST, Tsai SK, Chang YC, Wong CS. Intrathecal interleukin-1beta administration induces thermal hyperalgesia by activating inducible nitric oxide synthase expression in the rat spinal cord. Brain Res 2004; 1015: 145–53.

47 Lin Q, Wu J, Peng YB, Cui M, Willis WD. Nitric oxide-mediated spinal disinhibition contributes to the sensitization of primate spinothalamic tract neurons. J Neurophysiol 1999; 81: 1086–94.

48 Mantle D, Preedy VR. Free radicals as mediators of alcohol toxicity. Adverse Drug React Toxicol Rev 1999; 18: 235–52.

49 Zima T, Fialova L, Mestek O, Janebova M, Crkovska J, Malbohan I, Stipek S, Mikulikova L, Popov P. Oxidative stress, metabolism of ethanol and alcohol-related diseases. J Biomed Sci 2001; 8: 59–70.

50 Dicker E, Cederbaum AI. Increased NADH-dependent production of reactive oxygen intermediates by microsomes after chronic ethanol consumption: comparisons with NADPH. Arch Biochem Biophys 1992; 293: 274–80.

51 Mansouri A, Demeilliers C, Amsellem S, Pessayre D, Fromenty B. Acute ethanol administration oxidatively damages and depletes mitochondrial DNA in mouse liver, brain, heart, and skeletal muscles: protective effects of antioxidants. J Pharmacol Exp Ther 2001; 298: 737–43.

52 McDonough KH. Antioxidant nutrients and alcohol. Toxicology 2003; 189: 89–97.

53 Bosch-Morell F, Martinez-Soriano F, Colell A, Fernandez-Checa JC, Romero FJ. Chronic ethanol feeding induces cellular antioxidants decrease and oxidative stress in rat peripheral nerves: effect of *s*-adenosyl-l-methionine and *n*-acetyl-l-cysteine. Free Radic Biol Med 1998; 25: 365–68.

54 Tiwari V, Kuhad A, Chopra K. Amelioration of functional, biochemical and molecular deficits by epigallocatechin gallate in experimental model of alcoholic neuropathy. Eur J Pain 2011; 15: 286–92.

55 Tiwari V, Kuhad A, Chopra K. Tocotrienol ameliorates behavioral and biochemical alterations in the rat model of alcoholic neuropathy. Pain 2009; 145: 129–35.

56 Aley KO, Levine JD. Different peripheral mechanisms mediate enhanced nociception in metabolic/toxic and traumatic painful peripheral neuropathies in the rat. Neuroscience 2002; 111: 389–97.

57 Ahlgren SC, Levine JD. Protein kinase C inhibitors decrease hyperalgesia and C-fiber hyperexcitability in the streptozotocin-diabetic rat. J Neurophysiol 1994; 72: 684–92.

58 Taiwo YO, Bjerknes LK, Goetzl EJ, Levine JD. Mediation of primary afferent peripheral hyperalgesia by the cAMP second messenger system. Neuroscience 1989; 32: 577–80.

59 Khasar SG, Lin YH, Martin A, Dadgar J, McMahon T, Wang D, Hundle B, Aley KO, Isenberg W, McCarter G, Green PG, Hodge CW, Levine JD, Messing RO. A novel nociceptor signaling pathway revealed in protein kinase C epsilon mutant mice. Neuron 1999; 24: 253–60.

60 Aley KO, Martin A, McMahon T, Mok J, Levine JD, Messing RO. Nociceptor sensitization by extracellular signal-regulated kinases. J Neurosci 2001; 21: 6933–39.

61 Dina OA, McCarter GC, de Coupade C, Levine JD. Role of the sensory neuron cytoskeleton in second messenger signaling for inflammatory pain. Neuron 2003; 39: 613–24.

62 Dina OA, Hucho T, Yeh J, Malik-Hall M, Reichling DB, Levine JD. Primary afferent second messenger cascades interact with specific integrin subunits in producing inflammatory hyperalgesia. Pain 2005; 115: 191–203.

63 Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain 2005; 114: 149–59.

64 Dina OA, Aley KO, Isenberg W, Messing RO, Levine JD. Sex hormones regulate the contribution of PKCepsilon and PKA signalling in inflammatory pain in the rat. Eur J Neurosci 2001; 13: 2227–33.

65 Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, Watkins LR. Minocycline attenuates mechanical

allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain 2005; 115: 71–83.

66 Watkins LR, Milligan ED, Maier SF. Spinal cord glia: new players in pain. Pain 2001; 93: 201–5.

67 Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature 2001; 413: 203–10.

68 Norenberg ND. Astrocyte responses to CNS injury. J Neuropathol Exp Neurol 1994; 53: 213–20.

69 Robbins MA, Maksumova L, Pocock E, Chantler JK. Nuclear factor-kappaB translocation mediates double-stranded ribonucleic acid-induced NIT-1 beta-cell apoptosis and up-regulates caspase-12 and tumor necrosis factor receptor-associated ligand (TRAIL). Endocrinology 2003; 144: 4616–25.

70 Krebs JF, Armstrong RC, Srinivasan A, Aja T, Wong AM, Aboy A, Sayers R, Pham B, Vu T, Hoang K, Karanewsky DS, Leist C, Schmitz A, Wu JC, Tomaselli KJ, Fritz LC. Activation of membrane-associated procaspase-3 is regulated by Bcl-2. J Cell Biol 1999; 144: 915–26.

71 Joseph EK, Levine JD. Caspase signalling in neuropathic and inflammatory pain in the rat. Eur J Neurosci 2004; 20: 2896–902.

72 Jung ME, Gatch MB, Simpkins JW. Estrogen neuroprotection against the neurotoxic effects of ethanol withdrawal: potential mechanisms. Exp Biol Med (Maywood) 2005; 230: 8–22.

73 Izumi Y, Kitabayashi R, Funatsu M, Izumi M, Yuede C, Hartman RE, Wozniak DF, Zorumski CF. A single day of ethanol exposure during development has persistent effects on bi-directional plasticity, N-methyl-D-aspartate receptor function and ethanol sensitivity. Neuroscience 2005; 136: 269–79.

74 Meller ST, Dykstra C, Gebhart GF. Acute thermal hyperalgesia in the rat is produced by activation of *N*-methyl d-aspartate receptors and protein kinase C and production of nitric oxide. Neuroscience 1996; 71: 327–35.

75 Young MR, Fleetwood-Walker SM, Dickinson T, Blackburn-Munro G, Sparrow H, Birch PJ, Bountra C. Behavioural and electrophysiological evidence supporting a role for group I metabotropic glutamate receptors in the mediation of nociceptive inputs to the rat spinal cord. Brain Res 1997; 777: 161–9.

76 Jia H, Rustioni A, Valtschanoff JG. Metabotropic glutamate receptors in superficial laminae of the rat dorsal horn. J Comp Neurol 1999; 410: 627–42.

77 Valerio A, Paterlini M, Boifava M, Memo M, Spano P. Metabotropic glutamate receptor mRNA expression in rat spinal cord. Neuroreport 1997; 8: 2695–9.

78 Hudson LJ, Bevan S, McNair K, Gentry C, Fox A, Kuhn R, Winter J. Metabotropic glutamate receptor 5 upregulation in A-fibers after spinal nerve injury: 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reverses the induced thermal hyperalgesia. J Neurosci 2002; 22: 2660–8.

79 al-Ghoul WM, Volsi GL, Weinberg RJ, Rustioni A. Glutamate immunocytochemistry in the dorsal horn after injury or stimulation of the sciatic nerve of rats. Brain Res Bull 1993; 30: 453–59.

80 Miyoshi K, Narita M, Narita M, Suzuki T. Involvement of mGluR5 in the ethanol-induced neuropathic pain-like state in the rat. Neurosci Lett 2006; 410: 105–9.

81 Tracey DJ, Cunningham JE, Romm MA. Peripheral hyperalgesia in experimental neuropathy: mediation by alpha 2-adrenoreceptors on postganglionic sympathetic terminals. Pain 1995; 60: 317–27.

82 Singh B, Moodley J, Shaik AS, Robbs JV. Sympathectomy for complex regional pain syndrome. J Vasc Surg 2003; 37: 508–11.

83 Wang S, Lim G, Zeng Q, Sung B, Ai Y, Guo G, Yang L, Mao J. Expression of central glucocorticoid receptors after peripheral nerve injury contributes to neuropathic pain behaviors in rats. J Neurosci 2004; 24: 8595–605.

84 Dina OA, Khasar SG, Alessandri-Haber N, Green PG, Messing RO, Levine JD. Alcohol-induced stress in painful alcoholic neuropathy. Eur J Neurosci 2008; 27: 83–92.

85 Quintero L, Cuesta MC, Silva JA, Arcaya JL, Pinerua-Suhaibar L, Maixner W, Suarez-Roca H. Repeated swim stress increases pain induced expression of c-Fos in the rat lumbar cord. Brain Res 2003; 965: 259–68.

86 Khasar SG, Green PG, Levine JD. Repeated sound stress enhances inflammatory pain in the rat. Pain 2005; 116: 79–86.

87 Yerdelen D, Koc F, Uysal H. Strength-duration properties of sensory and motor axons in alcoholic polyneuropathy. Neurol Res 2008; 30: 746–50.

88 McLane JA. Decreased axonal transport in rat nerve following acute and chronic ethanol exposure. Alcohol 1987; 4: 385–89.

89 Hellweg R, Baethge C, Hartung HD, Brückner MK, Arendt T. NGF level in the rat sciatic nerve is decreased after long-term consumption of ethanol. Neuroreport 1996; 7: 777–80.

90 Malatova Z, Cizkova D. Effect of ethanol on axonal transport of cholinergic enzymes in rat sciatic nerve. Alcohol 2002; 26: 115–20.

91 Saunders DE, DiCerbo JA, Williams JR, Hannigan JH. Alcohol reduces neurofilament protein levels in primary cultured hippocampal neurons. Alcohol 1997; 14: 519–26.

92 Guru SC, Shetty KT, Shankar SK. Effect of chronic ethanol ingestion on phosphate content of neurofilament proteins and neurofilament associated protein phosphatase in rat spinal cord. Neurochem Res 1991; 16: 1193–97.

93 Ahluwalia B, Ahmad S, Adeyiga O, Wesley B, Rajguru S. Low levels of ethanol stimulate and high levels decrease phosphorylation in microtubule-associated proteins in rat brain: an in vitro study. Alcohol Alcohol 2000; 35: 452–57.

94 Kannarkat GT, Tuma DJ, Tuma PL. Microtubules are more stable and more highly acetylated in ethanol-treated hepatic cells. J Hepatol 2006; 44: 963–70.

95 Depaz IM, de Las Heras R, Kroon PA, Wilce PA. Changes in neuronal protein 22 expression and cytoskeletal association in the alcohol-dependent and withdrawn rat brain. J Neurosci Res 2005; 81: 253–60.

96 Netzel M, Ziems M, Jung KH, Noll E, Borsch C, Bitsch I. Effect of high-dosed thiamine hydrochloride and S-benzoylthiamine- O-monophosphate on thiamine-status after chronic ethanol administration. Biofactors 2000; 11: 111–3.

97 Woelk H, Lehrl S, Bitsch R, Kopcke W. Benfotiamine in treatment of alcoholic polyneuropathy: an 8-week randomized controlled study (BAP I Study). Alcohol Alcohol 1998; 33: 631–38.

98 Anisimova EI, Danilov AB. Benfotiamine efficacy in alcoholic polyneuropathy therapy. Zh Nevrol Psikhiatr Im S S Korsakova 2001; 101: 32–6.

99 Kishi Y, Schmelzer JD, Yao JK, Zollman PJ, Nickander KK, Tritschler HJ, Low PA. Alpha-lipoic acid: effect on glucose uptake, sorbitol pathway, and energy metabolism in experimental diabetic neuropathy. Diabetes 1999; 48: 2045–51.

100 Stevens MJ, Obrosova I, Cao X, Van Huysen C, Greene DA. Effects of DL-alpha-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. Diabetes 2000; 49: 1006–15.

101 Nagamatsu M, Nickander KK, Schmelzer JD, Raya A, Wittrock DA, Tritschler H, Low PA. Lipoic acid improves nerve blood flow, reduces oxidative stress, and improves distal nerve conduction in experimental diabetic neuropathy. Diabetes Care 1995; 18: 1160–67.

102 Maestri A, De Pasquale Ceratti A, Cundari S, Zanna C, Cortesi E, Crinò L. A pilot study on the effect of acetyl-L-carnitine in paclitaxel- and cisplatin-induced peripheral neuropathy. Tumori 2005; 91: 135–38.

103 Pisano C, Pratesi G, Laccabue D, Zunino F, Lo Giudice P, Bellucci A, Pacifici L, Camerini B, Vesci L, Castorina M, Cicuzza S, Tredici G, Marmiroli P, Nicolini G, Galbiati S, Calvani M, Carminati P, Cavaletti G. Paclitaxel and cisplatin-induced neurotoxicity: a protective role of acetyl-L-carnitine. Clin Cancer Res 2003; 9: 5756–67.

104 Tutuncu NB, Bayraktar M, Varli K. Reversal of defective nerve conduction with vitamin E supplementation in type 2 diabetes: a preliminary study. Diabetes Care 1998; 21: 1915–18.

105 Nickander KK, Schmelzer JD, Rohwer DA, Low PA. Effect of alpha-tocopherol deficiency on indices of oxidative stress in normal and diabetic peripheral nerve. J Neurol Sci 1994; 126: 6–14.

106 Saperstein DS, Barohn RJ. Peripheral neuropathy due to cobalamin deficiency. Curr Treat Options Neurol 2002; 4: 197–01.

107 Weir DG, Scott JM. The biochemical basis of the neuropathy in cobalamin deficiency. Baillieres Clin Haematol 1995; 8: 479–97.

108 Sun Y, Lai MS, Lu CJ. Effectiveness of vitamin B12 on diabetic neuropathy: systematic review of clinical controlled trials. Acta Neurol Taiwan 2005; 14: 48–54.

109 Sundkvist G, Dahlin LB, Nilsson H, Eriksson KF, Lindgärde F, Rosén I, Lattimer SA, Sima AA, Sullivan K, Greene DA. Sorbitol and myo-inositol levels and morphology of sural nerve in relation to peripheral nerve function and clinical neuropathy in men with diabetic, impaired, and normal glucose tolerance. Diabet Med 2000; 17: 259–68.

110 Carrington AL, Calcutt NA, Ettlinger CB, Gustafsson T, Tomlinson DR. Effects of treatment with myo-inositol or its 1,2,6-trisphosphate (PP56) on nerve conduction in streptozotocin-diabetes. Eur J Pharmacol 1993; 237: 257–63.

111 Love A, Cotter MA, Cameron NE. Effects of the sulphydryl donor N-acetyl-L-cysteine on nerve conduction, perfusion, maturation and regeneration following freeze damage in diabetic rats. Eur J Clin Invest 1996; 26: 698–706.

112 Park SA, Choi KS, Bang JH, Huh K, Kim SU. Cisplatin-induced apoptotic cell death in mouse hybrid neurons is blocked by antioxidants through suppression of cisplatin-mediated accumulation of p53 but not of Fas/Fas ligand. J Neurochem 2000; 75: 946–53.

113 Rains C, Bryson HM. Topical capsaicin. A review of its pharmacological properties and therapeutic potential in post-herpetic neuralgia, diabetic neuropathy and osteoarthritis. Drugs Aging 1995; 7: 317–28.

114 No authors listed. Treatment of painful diabetic neuropathy with topical capsaicin. A multicenter, double-blind, vehicle-controlled study. The Capsaicin Study Group. Arch Intern Med 1991; 151: 2225–9.

115 Finnerup NB, Otto M, McQuay HJ, Jensen TS, Sindrup SH. Algorithm for neuropathic pain treatment: an evidence based proposal. Pain 2005; 118: 289–305.

116 Rintala DH, Holmes SA, Courtade D, Fiess RN, Tastard LV, Loubser PG. Comparison of the effectiveness of amitriptyline and gabapentin on chronic neuropathic pain in persons with spinal cord injury. Arch Phys Med Rehabil 2007; 88: 1547–60.

117 Rowbotham MC, Goli V, Kunz NR, Lei D. Venlafaxine extended release in the treatment of painful diabetic neuropathy: a double-blind, placebo-controlled study. Pain 2004; 110: 697–06.

118 Raskin J, Pritchett YL, Wang F, D'Souza DN, Waninger AL, Iyengar S, Wernicke JF. A double-blind, randomized multicenter trial comparing duloxetine with placebo in the management of diabetic peripheral neuropathic pain. Pain Med 2005; 6: 346–56.

119 Sindrup SH, Bjerre U, Dejgaard A, Brøsen K, Aaes-Jørgensen T, Gram LF. The selective serotonin reuptake inhibitor citalopram relieves the symptoms of diabetic neuropathy. Clin Pharmacol Ther 1992; 52: 547–52.

120 Sandercock D, Cramer M, Wu J, Chiang YK, Biton V, Heritier M. Gabapentin extended release for the treatment of painful diabetic peripheral neuropathy: efficacy and tolerability in a double-blind, randomized, controlled clinical trial. Diabetes Care 2009; 32: e20.

121 Vestergaard K, Andersen G, Gottrup H, Kristensen BT, Jensen TS. Lamotrigine for central poststroke pain: a randomized controlled trial. Neurology 2001; 56: 184–90.

122 Eisenberg E, Lurie Y, Braker C, Daoud D, Ishay A. Lamotrigine reduces painful diabetic neuropathy: a randomized, controlled study. Neurology 2001; 57: 505–09.

123 Silver M, Blum D, Grainger J, Hammer AE, Quessy S. Double-blind, placebo-controlled trial of lamotrigine in combination with other medications for neuropathic pain. J Pain Symptom Manage 2007; 34: 446–54.

124 Rao RD, Flynn PJ, Sloan JA, Wong GY, Novotny P, Johnson DB, Gross HM, Renno SI, Nashawaty M, Loprinzi CL. Efficacy of lamotrigine in the management of chemotherapy-induced peripheral neuropathy: a phase 3 randomized, double-blind, placebo-controlled trial, N01C3. Cancer 2008; 112: 2802–8.

125 Kochar DK, Jain N, Agarwal RP, Srivastava T, Agarwal P, Gupta S. Sodium valproate in the management of painful neuropathy in type 2 diabetes – a randomized placebo controlled study. Acta Neurol Scand 2002; 106: 248–52.

126 Kochar DK, Rawat N, Agrawal RP, Vyas A, Beniwal R, Kochar SK, Garg P. Sodium valproate for painful diabetic neuropathy: a randomized double-blind placebo-controlled study. QJM 2004; 97: 33–8.

127 Kochar DK, Garg P, Bumb RA, Kochar SK, Mehta RD, Beniwal R, Rawat N. Divalproex sodium in the management of post-herpetic neuralgia: a randomized double-blind placebo-controlled study. QJM 2005; 98: 29–34.

128 Otto M, Bach FW, Jensen TS, Sindrup SH. Valproic acid has no effect on pain in polyneuropathy: a randomized, controlled trial. Neurology 2004; 62: 285–8.

129 Drewes AM, Andreasen A, Poulsen LH. Valproate for treatment of chronic central pain after spinal cord injury. A double-blind cross-over study. Paraplegia 1994; 32: 565–69.

130 Rauck RL, Shaibani A, Biton V, Simpson J, Koch B. Lacosamide in painful diabetic peripheral neuropathy: a phase 2 double-blind placebo-controlled study. Clin J Pain 2007; 23: 150–8.

131 Wymer JP, Simpson J, Sen D, Bongardt S, Lacosamide SP742 Study Group. Efficacy and safety of lacosamide in diabetic neuropathic pain: an 18-week double-blind placebo-controlled trial of fixed-dose regimens. Clin J Pain 2009; 25: 376–85.

132 Ziegler D, Hidvégi T, Gurieva I, Bongardt S, Freynhagen R, Sen D, Sommerville K, Lacosamide SP743 Study Group. Efficacy and safety of lacosamide in painful diabetic neuropathy. Diabetes Care 2010; 33: 839–41.