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Intralipid Infusion for Myelin Sheath Repair in Multiple Sclerosis and Trigeminal Neuralgia?

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Abstract

Multiple sclerosis is a demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged. Trigeminal neuralgia involves loss of the myelin around the trigeminal nerve. Myelin is a phospholipid membrane that wraps around axons to provide them with insulation. It is produced by Schwann cells in the PNS, and by oligodendrocytes in the CNS.

Intralipid treatment is first suggested in the medical literature as a way for myelin sheath repair in multiple sclerosis and trigeminal neuralgia.

Keywords: Intralipid, Fat Emulsion, Myelin, Multiple Sclerosis, Trigeminal Neuralgia, Mitochondria.

Multiple Sclerosis

Multiple sclerosis (MS) is a demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged [1]. This damage disrupts the ability of parts of the nervous system to communicate, resulting in a range of signs and symptoms, including physical, mental, and sometimes psychiatric problems [2-4]. Specific symptoms can include double vision, blindness in one eye, muscle weakness, trouble with sensation, or trouble with coordination [1]. MS takes several forms, with new symptoms either occurring in isolated attacks (relapsing forms) or building up over time (progressive forms) [5]. Between attacks, symptoms may disappear completely; however, permanent neurological problems often remain, especially as the disease advances [5].

Trigeminal Neuralgia

Trigeminal neuralgia (TN), also called tic douloureux, is a chronic pain condition that affects the trigeminal or 5th cranial nerve, one of the most widely distributed nerves in the head. TN is a form of neuropathic pain (pain associated with nerve injury or nerve lesion.) The typical or "classic" form of the disorder (called "Type 1" or TN1) causes extreme, sporadic, sudden burning or shock-like facial pain that lasts anywhere from a few seconds to as long as two minutes per episode. These attacks can occur in quick succession, in volleys lasting as long as two hours. The "atypical" form of the disorder (called "Type 2" or TN2), is characterized by constant aching, burning, stabbing pain of somewhat lower intensity than Type 1. Both forms of pain may occur in the same person, sometimes at the same time. The intensity of pain can be physically and mentally incapacitating.

The trigeminal nerve is one of 12 pairs of nerves that are attached to the brain. The nerve has three branches that conduct sensations from the upper, middle, and lower portions of the face, as well as the oral cavity, to the brain. The ophthalmic, or upper, branch supplies sensation to most of the scalp, forehead, and front of the head. The maxillary, or

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middle, branch stimulates the cheek, upper jaw, top lip, teeth and gums, and to the side of the nose. The mandibular, or lower, branch supplies nerves to the lower jaw, teeth and gums, and bottom lip. More than one nerve branch can be affected by the disorder. Rarely, both sides of the face may be affected at different times in an individual, or even more rarely at the same time (called bilateral TN).

TN is associated with a variety of conditions. TN can be caused by a blood vessel pressing on the trigeminal nerve as it exits the brain stem. This compression causes the wearing away or damage to the protective coating around the nerve (the myelin sheath). TN symptoms can also occur in people with multiple sclerosis, a disease that causes deterioration of the trigeminal nerve's myelin sheath. Rarely, symptoms of TN may be caused by nerve compression from a tumor, or a tangle of arteries and veins called an arteriovenous malformation. Injury to the trigeminal nerve (perhaps the result of sinus surgery, oral surgery, stroke, or facial trauma) may also produce neuropathic facial pain [6].

Current Treatment in Multiple Sclerosis and Trigeminal Neuralgia

Although many patients with multiple sclerosis (MS) complain of trigeminal neuralgia (TN), its cause and mechanisms are still debatable. In a multicentre controlled study, we collected 130 patients with MS:50 patients with TN, 30 patients with trigeminal sensory disturbances other than TN (ongoing pain, dysaesthesia, or hypoesthesia), and 50 control patients [7]. All patients underwent pain assessment, trigeminal reflex testing, and dedicated MRI scans. The MRI scans were imported and normalised into a voxel-based, 3D brainstem model that allows spatial statistical analysis. The onset ages of MS and trigeminal symptoms were significantly older in the TN group. The frequency histogram of onset age for the TN group showed that many patients fell in the age range of classic TN. Most patients in TN and non-TN groups had abnormal trigeminal reflexes. In the TN group, 3D brainstem analysis showed an area of strong probability of lesion ($P<0.0001$) centred on the intrapontine trigeminal primary afferents. In the non-TN group, brainstem lesions were more scattered, with the highest probability for lesions ($P<0.001$) in a region involving the sub nucleus oralis of the spinal trigeminal complex. We conclude that the most likely cause of MS-related TN is a pontine plaque damaging the primary afferents [7]. Nevertheless, in some patients a neurovascular contact may act as a concurring mechanism. The other sensory disturbances, including ongoing pain and dysaesthesia, may arise from damage to the second-order neurons in the spinal trigeminal complex [7].

Cryoneuroablation, also known as cryoanalgesia or cryoneurolysis, is a specialized technique for providing long-term pain relief in interventional pain management settings. Modern cryoanalgesia traces its roots to Cooper et al who developed in 1961, a device that used liquid nitrogen in a hollow tube that was insulated at the tip and achieved a temperature of-190 degrees C. Lloyd et al proposed that cryoanalgesia was superior to other methods of peripheral nerve destruction, including alcohol neurolysis, phenol neurolysis, or surgical lesions. The application of cold to tissues creates a conduction block, similar to the effect of local anesthetics. Long-term pain relief from nerve freezing occurs because ice crystals create vascular damage to the vasonervorum, which produces severe endoneural edema. Cryoanalgesia disrupts the nerve structure and creates wallerian degeneration but leaves the myelin sheath and endoneurium intact. Clinical applications of cryoanalgesia extend from its use in craniofacial pain secondary to trigeminal neuralgia, posterior auricular neuralgia, and glossopharyngeal neuralgia; chest wall pain with multiple conditions including post-thoracotomy neuromas, persistent pain after rib fractures, and post herpetic neuralgia in thoracic distribution; abdominal and pelvic pain secondary to ilioinguinal, iliohypogastric, genitofemoral, subgastric neuralgia; pudendal neuralgia; low back pain and lower extremity pain secondary to lumbar facet joint pathology, pseudosciatica, pain involving intraspinous ligament or supragluteal nerve, sacroiliac joint pain, cluneal neuralgia, obturator neuritis, and various types of peripheral neuropathy; and upper extremity pain secondary to suprascapular neuritis and other conditions of peripheral neuritis [8].

Patients with trigeminal neuralgia (TN) and multiple sclerosis (MS) are often treated with medications or a surgical procedure. However, there is little evidence that such treatments result in 50% pain reduction and improvement in quality of life. We searched Medline, EMBASE, and the Cochrane Collaboration database from inception until October 2016 [9]. Two authors independently selected studies for inclusions, data extraction and bias assessment.

All studies were of low quality using the GRADE system. For medical management, ten studies were included of which one was a randomised control trials. Two studies were on the use of misopropol, unique to patients with MS. For surgical therapy, 26 studies with at least 10 patients and a minimum of two-year follow-up were included. All types of surgical procedures are reported, and the results are poorer than for TN without MS with 50% having a recurrence by two years. The main complications were sensory loss. Many patients had to undergo further procedures to become pain free and there were no agreed prognostic factors.

There was insufficient evidence to support any one medical therapy and so earlier surgery may be preferable. A patient with TN and MS has therefore to make a decision based on low level evidence beginning with standard drug therapy and then choosing a surgical procedure [9].

Trigeminal neuralgia was defined by the International Association for the Study of Pain as a sudden, usually unilateral, severe, brief, stabbing recurrent pain in the distribution of one or more branches of the fifth cranial nerve. Standard treatment is with anti-epileptic drugs. Non-antiepileptic drugs have been used in the management of trigeminal neuralgia since the 1970s. This is an update of a review first published in 2006 and previously updated in 2011.

To systematically review the efficacy and tolerability of non-antiepileptic drugs for trigeminal neuralgia. On 20 May

2013, for this updated review, we searched the Cochrane Neuromuscular Disease Group Specialized Register, CENTRAL (2013, Issue 4), MEDLINE (January 1966 to May 2013), EMBASE (January 1980 to May 2013), LILACS (January 1982 to May 2013) and the Chinese Biomedical Retrieval System (1978 to May 2013) [10]. We searched clinical trials registries for ongoing trials. We included double-blind, randomised controlled trials in which the active drug was used either alone or in combination with other non-antiepileptic drugs for at least two weeks. Two authors decided which trials fitted the inclusion criteria and independently graded risk of bias. We assessed the quality of the evidence according to the GRADE criteria for this update.

In this 2013 update, we updated the searches, but identified only two new ongoing studies. The review includes four trials involving 139 participants. The primary outcome measure in each was pain relief. Three trials compared one of the oral non-antiepileptic drugs tizanidine, tocainide or pimozide with carbamazepine. The quality of evidence for all outcomes for which data were available was low. In a trial of tizanidine involving 12 participants (one dropped out due to unrelated disease), one of five participants treated with tizanidine and four of six treated with carbamazepine improved (risk ratio (RR) 0.30, 95% confidence interval (CI) 0.05 to 1.89). Few side effects were noted with tizanidine. For pimozide, there was evidence of greater efficacy than carbamazepine at six weeks. Up to 83% of participants reported adverse effects but these did not lead to withdrawal; the report did not provide comparable data for carbamazepine. Limited data meant that we could not assess the effects of tocainide; however, data from non-randomised studies (not included in this review) indicate that serious haematological adverse events can occur. A trial involving 47 participants compared 0.5% proparacaine hydrochloride eyedrops with placebo but did not show any significant benefits, again according to low-quality evidence. The report did not mention adverse events. The proparacaine trial was at low risk of bias; the other trials were at unclear risk of bias overall.

There is low-quality evidence that the effect of tizanidine is not significantly different than that of carbamazepine in treating trigeminal neuralgia. Pimozide is more effective than carbamazepine, although the evidence is of low quality and the data did not allow comparison of adverse event rates. There is also low-quality evidence that 0.5% proparacaine hydrochloride eye drops have no benefit over placebo. Limitations in the data for tocainide prevent any conclusions being drawn. There is insufficient evidence from randomised controlled trials to show significant benefit from non-antiepileptic drugs in trigeminal neuralgia. More research is needed [10].

Neuromodulation, or the utilization of advanced technology for targeted electrical or chemical neuronal stimulation or inhibition, has been expanding in several neurological subspecialties. In the past decades, immune-modulating therapy has been the main focus of multiple sclerosis (MS) research with little attention to neuromodulation. However, with the recent advances in disease-modifying therapies, it is time to shift the focus of MS research to neuromodulation and restoration of function as with other neurological subspecialties. Preliminary research supports the value of intrathecal baclofen pump and functional electrical stimulation in improving spasticity and motor function in MS patients. Deep brain stimulation can improve MS-related tremor and trigeminal neuralgia. Spinal cord stimulation has been shown to be effective against MS-related pain and bladder dysfunction. Bladder overactivity also responds to sacral neuromodulation and posterior tibial nerve stimulation. Despite limited data in MS, transcranial magnetic stimulation and brain-computer interface are promising neuromodulatory techniques for symptom mitigation and neurorehabilitation of MS patients [11].

Trigeminal neuralgia (TN) is a sudden, severe, brief, stabbing, and recurrent pain within one or more branches of the trigeminal nerve. Type 1 as intermittent and Type 2 as constant pain represent distinct clinical, pathological, and prognostic entities.

Although multiple mechanism involving peripheral pathologies at root (compression or traction), and dysfunctions of brain stem, basal ganglion, and cortical pain modulatory mechanisms could have role, neurovascular conflict is the most accepted theory.

Diagnosis is essentially clinically; magnetic resonance imaging is useful to rule out secondary causes, detect pathological changes in affected root and neurovascular compression (NVC). Carbamazepine is the drug of choice; oxcarbazepine, baclofen, lamotrigine, phenytoin, and topiramate are also useful. Multidrug regimens and multidisciplinary approaches are useful in selected patients. Microvascular decompression is surgical treatment of choice in TN resistant to medical management. Patients with significant medical comorbidities, without NVC and multiple sclerosis are generally recommended to undergo gamma knife radiosurgery, percutaneous balloon compression, glycerol rhizotomy, and radiofrequency thermocoagulation procedures. Partial sensory root sectioning is indicated in negative vessel explorations during surgery and large intraneural vein. Endoscopic technique can be used alone for vascular decompression or as an adjuvant to microscope. It allows better visualization of vascular conflict and entire root from pons to ganglion including ventral aspect. The effectiveness and completeness of decompression can be assessed and new vascular conflicts that may be missed by microscope can be identified. It requires less brain retraction [12].

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Of the plethora of motor and sensory disturbances experienced by sufferers, neuropathic pain is a highly prevalent and debilitating symptom, and at present remains extremely difficult to treat. Common forms of neuropathic pain seen in MS patients include central neuropathic pain, Lhermitte's phenomenon and trigeminal neuralgia, which are all speculated to arise from specific patterns of lesion formation.

Efficacious pharmacological interventions for the treatment of neuropathic pain associated with MS are lacking and have been largely informed by drug trials in peripheral neuropathies and spinal cord injury.

Neuropathic pain in MS is inadequately relieved by conventional analgesics, and first-line therapies are generally comprised of anti-depressive and anti-convulsive drugs. A range of alternatives have been proposed and tested with variable success, including cannabinoids and certain opioid analgesics. Animals with experimental autoimmune encephalomyelitis (EAE), an autoimmune model of MS, also exhibit neuropathic pain symptoms.

Studies aimed at understanding the mechanisms underlying EAE-induced neuropathic pain and investigating the efficacy of novel pharmacological interventions at the animal level offer an exciting area of future research and may inform future therapeutic options for MS-associated neuropathic pain [13].

Trigeminal neuralgia (TN) associated with multiple sclerosis (MS) was first described in Lehrbuch der Nervenkrankheiten für Ärzte und Studirende in 1894 by Hermann Oppenheim, including a pathologic description of trigeminal root entry zone demyelination. Early English-language translations in 1900 and 1904 did not so explicitly state this association compared with the German editions. The 1911 English-language translation described a more direct association. Other later descriptions were clinical with few pathologic reports, often referencing Oppenheim but citing the 1905 German or 1911 English editions of Lehrbuch. This discrepancy in part may be due to the translation differences of the original text [14].

Although trigeminal neuralgia is well known to neurologists, recent developments in classification and clinical diagnosis, new MRI methods, and a debate about surgical options necessitate an update on the topic.

Currently, a worldwide controversy exists regarding the classification, diagnostic process, and surgical treatment of trigeminal neuralgia. This controversy has been caused on one side by the recognition that some 50% of patients with trigeminal neuralgia, apart from characteristic paroxysmal attacks, also have continuous pain in the same territory, which results in greater diagnostic difficulties and is associated with a lower response to medical and surgical treatments. In contrast, recent developments in MRI methods allow differentiation between a mere neurovascular contact and an effective compression of the trigeminal root by an anomalous vessel, which implies more difficulties in the choice of surgical treatment, with the indication for microvascular decompression becoming more restricted.

This article proposes that the diagnosis of trigeminal neuralgia, with or without concomitant continuous pain, must rely on clinical grounds only [15]. Diagnostic tests are necessary to distinguish three etiologic categories: idiopathic trigeminal neuralgia (nothing is found), classic trigeminal neuralgia (an anomalous vessel produces morphologic changes of the trigeminal root near its entry into the pons), and secondary trigeminal neuralgia (due to major neurologic disease, such as multiple sclerosis or tumors at the cerebellopontine angle). Carbamazepine and oxcarbazepine (i.e. voltage-gated, frequency-dependent sodium channel blockers) are still the first-choice medical treatment, although many patients experience significant side effects, and those with concomitant continuous pain respond less well to treatment. The development of sodium channel blockers that are selective for the sodium channel 1.7 (Nav1.7) receptor will hopefully help. Although all the surgical interventions (percutaneous ganglion lesions, gamma knife radiosurgery, and microvascular decompression) are very efficacious, precise MRI criteria for differentiating a real neurovascular compression from an irrelevant contact will be of benefit in better selecting patients for microvascular decompression [15].

Myelin Sheath in Multiple Sclerosis

Myelin is a phospholipid membrane that wraps around axons to provide them with insulation. It is produced by Schwann cells in the PNS, and by oligodendrocytes in the CNS. Myelin clearance is the next step in Wallerian degeneration following axonal degeneration. The cleaning up of myelin debris is different for PNS and CNS. PNS is much faster and efficient at clearing myelin debris in comparison to CNS, and Schwann cells are the primary cause of this difference. Another key aspect is the change in permeability of the blood-tissue barrier in the two systems. In PNS, the permeability increases throughout the distal stump, but the barrier disruption in CNS is limited to just the site of injury [16].

Regeneration follows degeneration. Regeneration is rapid in PNS, allowing for rates of up to 1 millimeter a day of regrowth [17]. Grafts may also be needed to allow for appropriate reinnervation. It is supported by Schwann cells through growth factors release. CNS regeneration is much slower, and is almost absent in most vertebrate species. The primary cause for this could be the delay in clearing up myelin debris. Myelin debris, present in CNS or PNS, contains several inhibitory factors. The prolonged presence of myelin debris in CNS could possibly hinder the regeneration [18]. An experiment conducted on Newts, animals that have fast CNS axon regeneration capabilities, found that Wallerian degeneration of an optic nerve injury took up to 10 to 14 days on average, further suggesting that slow clearance inhibits regeneration [19].

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disorder of the CNS characterized by infiltration of immune cells and progressive damage to myelin sheaths and neurons. In recent years, the importance of the neuronal compartment in the early pathology of multiple sclerosis has become increasingly clear. Direct axonal damage within the early stages of inflammation as well as neuronal injury as a result of chronic demyelination are essential factors for the development of long-term disability in patients. Viewing MS as both inflammatory and neurodegenerative has significant implications for treatment, with remyelination of denuded axons to protect neurons from damage being necessary in addition to controlling inflammation [20].

This review, focused on demyelination in multiple sclerosis, is divided in two parts [21]. The first part addresses the many and not exclusive mechanisms leading to demyelination in the central nervous system. Although the hypothesis that a primary oligodendrocyte or myelin injury induces a secondary immune response in the central nervous system is still a matter of debate, most recent advances underline the influence of a primary immune response against myelin antigen(s), with a diversity of potential targets. Whereas multiple sclerosis was long considered as a T cell-mediated disease, the role of B lymphocytes is now increasingly recognized, and the influence of antibodies on tissue damage actively investigated. The second part of the review describes the axonal consequences of demyelination. Segmental demyelination results in conduction block or slowing of conduction through adaptative responses, notably related to modifications in the distribution of voltage gated sodium channels along the denuded axon. If demyelination persists, these changes, as well as the loss of trophic and metabolic support, will lead to irreversible axonal damage and loss. In this respect, favouring early myelin repair, during a window of time when axonal damage is still reversible, might pave the way for neuroprotection [21].

The myelin sheath that coats axons allows rapid propagation of electrical impulses across the nervous system. Oligodendrocytes (ODs) are myelin-producing cells of the central nervous system (CNS) responsible for wrapping the axons of neurons. Multiple sclerosis (MS) is a demyelinating disease of the CNS identifiable by white and grey matter lesions. These lesions consist of axons that have lost their myelin through an autoimmune response to myelin and ODs. Current treatments for MS target the autoimmune aspect of the disease. However, these immunomodulators do not directly enhance the process of remyelination. The ability to remyelinate lesions can be enhanced by neural progenitor cells that can differentiate into ODs and replace lost myelin, although successful remyelination is complex and dependent on multiple factors. The restoration of lost myelin might protect the axon from degeneration and restore optimal conduction of impulses in MS patients, requiring further research on proremyelinating therapies. The combination of immunomodulators and remyelinating enhancers might be the best course of treatment for many MS patients [22].

Progressive phases of multiple sclerosis are associated with inhibited differentiation of the progenitor cell population that generates the mature oligodendrocytes required for remyelination and disease remission. To identify selective inducers of oligodendrocyte differentiation, we performed an image-based screen for myelin basic protein (MBP) expression using primary rat optic-nerve-derived progenitor cells [23]. Here we show that among the most effective compounds identified was benztropine, which significantly decreases clinical severity in the experimental autoimmune encephalomyelitis (EAE) model of relapsing-remitting multiple sclerosis when administered alone or in combination with approved immunosuppressive treatments for multiple sclerosis. Evidence from a cuprizone-induced model of demyelination, *in vitro* and *in vivo* T-cell assays and EAE adoptive transfer experiments indicated that the observed efficacy of this drug results directly from an enhancement of remyelination rather than immune suppression. Pharmacological studies indicate that benztropine functions by a mechanism that involves direct antagonism of M1 and/or M3 muscarinic receptors. These studies should facilitate the development of effective new therapies for the treatment of multiple sclerosis that complement established immunosuppressive approaches [23].

Multiple sclerosis (MS) is widely considered to be the result of an aggressive autoreactive T cell attack on myelin. How these autoimmune responses arise in MS is unclear, but they could result from virus infections. Thus, viral and autoimmune diseases in animals have been used to investigate the possible pathogenic mechanisms operating in MS. The autoimmune model, experimental autoimmune encephalomyelitis, is the most widely-used animal model and has greatly influenced therapeutic approaches targeting autoimmune responses. To investigate demyelination and remyelination in the absence of the adaptive immune response, toxin-induced demyelination models are used. These include using cuprizone, ethidium bromide and lysolecithin to induce myelin damage, which rapidly lead to remyelination when the toxins are withdrawn. The virus models include natural and experimental infections such as canine distemper, visna infection of sheep, and infection of non-human primates. The most commonly used viral models in rodents are Semliki Forest virus and Theiler's murine encephalomyelitis virus. The viral and experimental autoimmune encephalomyelitis models have been instrumental in the understanding of how viruses trigger inflammation, demyelination and neurodegeneration in the central nervous system. However, due to complexity of the animal models, pathological mechanisms are also examined in central nervous system cell culture systems including co-cultures, aggregate cultures and brain slice cultures [24].

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system and the leading cause of non-traumatic neurologic disability in young adults in the United States and Europe. The disease course is variable and starts with reversible episodes of neurologic disability which transforms into continuous and irreversible neurologic decline. It is well established that loss of axons and neurons is the major cause of the progressive neurologic decline that most MS patients endure. Current hypotheses support primary inflammatory demyelination as the underlying cause of axonal loss during earlier stages in MS. The transition to progressive disease course is thought to occur when a threshold of neuronal and axonal loss is reached, and the compensatory capacity of the central nervous system is surpassed. Available immunomodulatory therapies are of little benefit to MS after entering this irreversible phase of the disease. Elucidation of mechanisms that are responsible for axonal loss is therefore essential for the development of therapies directed to stop neurologic decline in MS patients [25].

Demyelination and axonal damage are responsible for neurological deficits in multiple sclerosis (MS), an

inflammatory demyelinating disease of the central nervous system. However, the pathology of axonal damage in MS is not fully understood. In this study, histological analysis of morphological changes of axonal organelles during demyelination in murine models was investigated by scanning electron microscopy (SEM) using an osmium-maceration method. In cuprizone-induced demyelination, SEM showed typical morphology of demyelination in the corpus callosum of mouse brain. In contrast, SEM displayed variations in ultrastructural abnormalities of myelin structures and axonal organelles in spinal cord white matter of experimental autoimmune encephalomyelitis (EAE) mice, an animal model of MS. Myelin detachment and excessive myelin formation were observed as typical morphological myelin abnormalities in EAE. In addition, well-developed axoplasmic reticulum-like structures and accumulated mitochondria were observed in tortuous degenerating/degenerated axons and the length of mitochondria in axons of EAE spinal cord was shorter compared with naïve spinal cord. Immunohistochemistry also revealed dysfunction of mitochondrial fusion/fission machinery in EAE spinal cord axons. Moreover, the number of Y-shaped mitochondria was significantly increased in axons of the EAE spinal cord. Axonal morphologies in myelin basic protein-deficient shiverer mice were similar to those in EAE. However, shiverer mice had "tortuous" (S-curve shaped mitochondria) and larger mitochondria compared with wild-type and EAE mice. Lastly, analysis of human MS patient autopsied brains also demonstrated abnormal myelin structures in demyelinating lesions. These results indicate that morphological abnormalities of myelin and axonal organelles play important role on the pathogenesis of axonal injury in demyelinating [26].

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system characterized by infiltration of immune cells and progressive damage to myelin and axons. All therapeutics used to treat MS have been developed to target an overactive immune response, with aims to reduce disease activity. Chronic demyelinated axons are further prone to irreversible damage and death, and it is imperative that new therapies address this critical issue. Remyelination, the generation of new myelin in the adult nervous system, is an endogenous repair mechanism that restores function of denuded axons and delays their deterioration. Although remyelination can be extensive in some patients, the majority of cases limit repair only to the acute phase of disease. A significant current drive in new MS therapeutics is to identify targets that can promote remyelination by boosting endogenous oligodendrocyte precursor cells to form new myelin. Also, a number of inhibitory pathways have been identified in chronic MS lesions that prevent oligodendrocyte precursor cells from being properly recruited to demyelinated lesions or interfere with their differentiation to myelin-forming oligodendrocytes [27].

Pathological examination of the affected human tissue is key to understanding the possible mechanisms operating in the disease. In multiple sclerosis (MS), studies of central nervous system (CNS) tissues reveal the inflammatory nature of the disease associated with demyelination and axonal damage. Based on the concept of a pathogenic adaptive immune response, immunosuppressive therapies have been developed in an attempt to block or inhibit the potentially pathogenic T and B cells. More recently, re-examination of the neuropathology has led to a resurgence of interest in the neurodegenerative aspects of the disease, the involvement of cortical damage as well as the role of innate immunity in MS. These ideas have led to paradigm shifts from MS being the result of autoimmunity to myelin due to initial adaptive immune responses, to that of a neurodegenerative disease in which, besides T and B cells, innate immunity may play a major role in the disease process. The neuropathological studies have undoubtedly influenced pharmaceutical interest in development of neuroprotective approaches [28].

Multiple sclerosis (MS) patients today have more hope of a good disease outcome with an ever-increasing choice of immunomodulatory therapies to reduce disease relapses, thought to be caused by inflammation within the CNS, leading to axonal demyelination.

However, although there has been much progress in this disease phase, there has been little impact on the progressive phase of MS, when neurodegeneration dominates, and patients accumulate disability over years. This failure of prevention of progressive disease has led to a frame-shift in research thinking, focusing on neuroprotective strategies such as promotion of remyelination, to be used alongside immunomodulatory therapies [29].

Oligodendrocytes have limited ability to repair the damage to themselves or to other nerve cells, as seen in demyelinating diseases like multiple sclerosis. An important strategy may be to replace the lost oligodendrocytes and/or promote the maturation of undifferentiated oligodendrocyte precursor cells (OPCs). Recent studies show that a composite of co-ultramicronized N-palmitoylethanolamine (PEA) and luteolin (co-ultramicronized PEA/luteolin, 10:1 by mass) is efficacious in improving outcome in experimental models of spinal cord and traumatic brain injuries. Here, we examined the ability of co-ultramicronized PEA/luteolin to promote progression of OPCs into a more differentiated phenotype [30]. OPCs derived from newborn rat cortex were placed in culture and treated the following day with 10 μM co-ultramicronized PEA/luteolin. Cells were collected 1, 4 and 8 days later and analyzed for expression of myelin basic protein (MBP). qPCR and Western blot analyses revealed a time-dependent increase in expression of both mRNA for MBP and MBP content, along with an increased expression of genes involved in lipid biogenesis. Ultramicronized PEA or luteolin, either singly or in simple combination, were ineffective. Further, co-ultramicronized PEA/luteolin promoted morphological development of OPCs and total protein content without affecting proliferation. Co-ultramicronized PEA/luteolin may represent a novel pharmacological strategy to promote OPC maturation [30].

Myelin Sheath in Teigeminal Neuralgia

Neuropathic pain that is the chronic, severe, and intractable pain, interferes with activities of daily living (ADL) and consequently reduces quality of life (QOL). We reported the efficacy of Yokukansan in patients with

neuropathic pain, including acute herpetic pain, postherpetic neuralgia, central poststroke pain, post-traumatic spinal cord injury pain, thalamic syndrome, complex regional pain syndrome and symptomatic trigeminal neuralgia [31]. Yokukansan was more effective compared with traditional medicines, such as tricyclic antidepressants, carbamazepine, gabapentin, and opioids etc., which are recommended to treat neuropathic pain. Recently, effects of Yokukansan is reported on the behavioral and psychological symptoms of dementia (BPSD) in elderly patients with dementia. Repeated administration of Yokukansan decreases expression of 5-hydroxytryptamine (5-HT) 2A receptors in the prefrontal cortex in mice, and Yokukansan also protects destruction of myelin sheaths in rats with thiamine deficient-induced encephalopathy. Mechanism of effectiveness of Yokukansan on neuropathic pain has not been established; however, efficacy of Yokukansan on neuropathic pain has been shown clinically.

As far as we know, this is the first report that Yokukansan was effective on neuropathic pain. Yokukansan without serious adverse reactions may be a possible medicine for treatment of neuropathic pain in future [31].

Detailed ultrastructural and immunohistochemical examination of the trigeminal axons surrounded by the peripheral type of the myelin could add new information about the extent of the trigeminal nerve lesion in neuralgia.

The examination comprised, firstly, the 10 trigeminal nerve roots (TNRs) in which the neurovascular contact was found in 20% of the cases, and the 2 additional control TNRs. Secondly, the biopsy specimens were taken from 6 patients with trigeminal neuralgia and 2 patients with trigeminal neuropathy following a partial TNR rhizotomy. The specimens were examined under the electron microscope (EM) and/or using the immunohistochemical (IHC) methods.

In addition to the central zone of demyelination, the EM examination of the TNR also revealed alterations of the peripheral myelin, i.e. deformation, thickening, demyelination and remyelination, as well as changes of the peripheral axons, that is, atrophy or hypertrophy, neurofilaments increase, loss of the myelin and sprouting occasionally. Some Schwann cells were also damaged. The IHC examination usually showed a moderate immune reaction against neuron-specific enolase (NSE) and protein gene product 9.5 (PGP9.5), but sporadically weaker reaction against the S-100 protein, synaptophysin (SY), neurofilament protein (NFP) and glial fibrillary acidic protein (GFAP). The substance P (SP) and calcitonin gene-related peptide (CGRP) immunoreactivity was weak at some sites, but strong at some other places.

The pathological changes affect not only the central nerve fibers of the TNR, but also some of the peripheral axons, their myelin sheath and Schwann cells. These are signs of the retrograde ultrastructural and biochemical alterations, which could participate in the pathophysiological mechanism underlying the trigeminal neuralgia [32].

The aim of this study was to evaluate the microanatomy of the central myelin-peripheral myelin transitional zone (TZ) in trigeminal nerves from cadavers [33]. One hundred trigeminal nerves from 50 cadaver heads were examined. The cisternal portion of the nerve (from the pons to Meckel's cave) was measured. Horizontal sections were stained and photographed. The photomicrographs were used to measure the extent of central myelin on the medial and lateral aspects of the nerve and to classify TZ shapes.

The cisternal portions of the specimens ranged from 8 to 15 mm long (mean, 12.3 mm; median, 11.9 mm). The data from the photomicrographs revealed that the extent of central myelin (distance from pons to TZ) on the medial aspect of the nerve (range, 0.1-2.5 mm; mean, 1.13 mm; median, 1 mm) was shorter than that on the lateral aspect (range, 0.17-6.75 mm; mean, 2.47 mm; median, 2.12 mm).

The data definitively prove that the root entry zone (REZ, nerve-pons junction) and TZ of the trigeminal nerve are distinct sites and that these terms should never be used interchangeably. The measurements showed that the central myelin occupies only the initial one-fourth of the trigeminal nerve length. If trigeminal neuralgia is caused exclusively by vascular compression of the central myelin, the problem vessel would always have to be located in this region. However, it is well known that pain from trigeminal neuralgia can resolve after vascular decompression at more distal sites.

This suggests that the effects of surgical decompression are caused by another mechanism [33]. Trigeminal involvement detected by magnetic resonance imaging (MRI) in multiple sclerosis (MS) patients is usually associated with trigeminal neuralgia (TN) or painless paraesthesia in the trigeminal distribution. Our (34) aim is to review the incidence of trigeminal involvement on MRI in a series of patients with MS at our institution, with further clinical correlation. We reviewed MRI scans of 275 MS patients for the presence of gadolinium enhancement on postcontrast T1-weighted images, anatomical and signal abnormalities on different sequences at the pontine trigeminal root entry zone (REZ) and in the cisternal portion of the nerves. We observed enhancement in the cisternal portion of the nerves and signal abnormalities (with or without enhancement) at the pontine trigeminal REZ in 8(2.9%) patients, and enhancement was bilateral in 6(75%) of those. Despite the inflammatory activity, none of them had TN and 3(37.5%) had only painless paraesthesias in the correspondent V3 distribution. We also found a marked trigeminal hypertrophy in 2(25%) patients, both with a longer period of disease. Our results confirm a high and clinically silent incidence of trigeminal involvement in MS patients, and suggest a simultaneous role of the central and peripheral type of myelin in trigeminal demyelination [34].

We investigated the effects of percutaneous gasserian glycerol injection in dogs and reviewed the histopathological changes. Experiments were performed in 16 adult healthy mongrel dogs. In group 1 (8 dogs) normal saline and in group 2 (8 dogs) pure glycerol was injected in the right trigeminal ganglion [35]. After these procedures, dogs in each group were sacrificed after 24 h (3 dogs), 7 days (3 dogs), 21 days (2 dogs).

The trigeminal ganglion and nerve of both sides were removed by using microsurgical techniques and examined by light and electron microscopy. Group 1: in all sections, nerve cells, myelinated and nonmyelinated fibers revealed normal patterns with slight fibrosis. Group 2: in all sections, myelinated fibers showed disintegration and swelling of the myelin sheath, rupture of axon continuity, destruction of basal lamina, deformation of the myelin-axon relationship by both light microscopy and electron microscopy. The sections examined by electron microscopy also showed axonolysis in nonmyelinated fibers. The changes after 7 and 21 days were less prominent than after 24 h. In the left sides, there are no pathological changes. Glycerol has a neurolytic effect on the dog's trigeminal ganglion. These effects were not specific and selective for myelinated and nonmyelinated nerve fibers [35].

Recent progress in the understanding of abnormal electrical behavior in injured sensory neurons motivated an examination, at the ultrastructural level, of trigeminal roots of patients with trigeminal neuralgia (TN). In 12 patients biopsy specimens of trigeminal root were obtained during surgery for microvascular decompression. Pathological changes in tissue included axonopathy and axonal loss, demyelination, a range of less severe myelin abnormalities (dysmyelination), residual myelin debris, and the presence of excess collagen, including condensed collagen masses in two cases. Within zones of demyelination, groups of axons were often closely apposed without an intervening glial process. Pathological characteristics of nerve fibers were clearly graded with the degrees of root compression noted at operation. Pain also occurred, however, in some patients who did not appear to have a severe compressive injury.

Findings were consistent with the ignition hypothesis of TN. This model can be used to explain the major positive and negative symptoms of TN by axonopathy-induced changes in the electrical excitability of afferent axons in the trigeminal root and of neuronal somata in the trigeminal ganglion. The key pathophysiological changes include ectopic impulse discharge, spontaneous and triggered after discharge, and cross excitation among neighbouring afferents [36].

The aim of our study was to document whether relationships existed among bone morphogenetic proteins (BMPs), peripheral nerve and neoplastic lesions of nerve sheath tumors [37]. The mRNA transcriptions of BMP-2, 3, 4 and 5 in 10 cases of schwannoma, three cases of malignant schwannoma and two cases of trigeminal neuralgia were detected using an in-situ hybridization technique. Our results demonstrated that the myelin sheaths of Schwann cell from the peripheral neuroectomy of trigeminal neuralgia positively expressed mRNA of BMP-2, 3, 4, and 5. The most interesting finding was that the nerve fibers of trigeminal nerve showed only BMP-2 positive staining. All the neoplastic lesions of nerve sheath showed a consistent but variant expression of BMP-2, 3, 4, and 5. The expression signals of BMP-2, 3, 5 mRNA in malignant schwannoma were relatively lower than in benign lesions except for the expression of BMP-4 mRNA. Our results indicated that selected members of BMPs were expressed in the peripheral nerves that might contribute to the health maintenance, proliferation, regeneration and neoplastic transformation of the peripheral nerve system. Furthermore, the effects of BMP-2, 3, 4 and 5 on peripheral nervous system during neoplastic transformation might be widespread, diverse and antagonistic [37].

Trigeminal neuralgia is a well-recognized complication of multiple sclerosis. In patients with neuralgia not responding to medical treatment or transcutaneous ablative procedures, the pain can often be treated successfully by partial rhizotomy of the trigeminal sensory root. We have examined partial trigeminal rhizotomy specimens from six multiple sclerosis patients, aged between 34 and 77 years, with intractable trigeminal neuralgia lasting between 18 months and 11 years [38]. The rhizotomy specimens were placed in buffered glutaraldehyde immediately after resection, and subsequently processed for electron microscopy. In all cases, this revealed demyelination in the proximal (CNS) part of the nerve root, with associated gliosis and variable inflammation. A consistent feature was the presence of clusters of juxtaposed axons without intervening glial processes. Similar juxtaposition of axons was previously observed in trigeminal neuralgia due to vascular compression of the nerve root. Experimental studies indicate that this arrangement of demyelinated axons is conducive to both spontaneous impulse activity and ephaptic spread of excitation. The demyelination and associated juxtaposition of axons may therefore account for key aspects of the pathogenesis of trigeminal neuralgia [38].

Bone morphogenetic proteins (BMPs) have been shown to play an important role in cell growth and differentiation. BMPs, a rapidly expanding family closely related to transforming growth factor-beta (TGF-beta) superfamily, have been proven recently to possess a regulatory role and neurotrophic capacity in neurogenesis. The aim of the present study is to reveal the relationship among BMPs, peripheral nerve and neoplastic lesions of nerve sheath tumors. The mRNA transcriptions of BMP 2, 3, 4 and 5 in 12 cases of schwannoma, four cases of malignant schwannoma and three cases of trigeminal neuralgia were detected using an in-situ hybridization technique. Our results demonstrated that the myelin sheaths of schwann cell from the peripheral neuroectomy of trigeminal neuralgia were positively expressing mRNA of BMP-2, 3, 4 and 5. However, the nerve fibers of trigeminal nerve showed only BMP-2 positive staining. All of the neoplastic lesions of nerve sheath showed a consistent but variant expression of BMP-2, 3, 4, and 5. Except for the BMP-4 mRNA, the expression signals of BMP-2, 3 and 5 mRNA in malignant schwannoma were relatively lower than in benign lesions. On the basis of the findings, we concluded that selected members of BMPs existed in the peripheral nerves and might contribute to the health maintenance, proliferation, regeneration and neoplastic transformation of the peripheral nerve system. Moreover, the effects of BMP-2, 3, 4 and 5 on peripheral nerve system and its neoplastic transformation might be widespread, diverse and antagonistic [39].

We have examined the ultrastructure of the trigeminal sensory nerve root in three patients with medically intractable trigeminal neuralgia [40]. In one patient, the nerve root was sandwiched between a large vein and a small pontine artery, in the others compression was due to marked dolichoectasia of a vertebral artery. Because these were not amenable to microvascular decompression, a caudal rhizotomy was performed, by excising a short inferior segment of nerve root in the region of indentation. In all cases, examination revealed a zone of chronic demyelination in the proximal (centrally myelinated) part of the root, near its junction with peripheral nerve. The zone of demyelination contained closely packed axons without intervening glial cytoplasm. Also present were small numbers of thinly myelinated axons. In some cases, a single thin myelin sheath encircled several adjacent axons that were still in close apposition. These findings indicate that the trigeminal neuralgia associated with vascular compression is due to demyelination. The demyelination is associated with some evidence of remyelination. The latter phenomenon may account in part for the long periods of remission, especially during the initial period after the onset of trigeminal neuralgia. The partly aberrant nature of the myelination within the region of vascular compression may contribute to the persistence of symptoms in some patients after decompressive surgery [40].

Glycerol was injected into the infraorbital canal of 12 rats to determine neurolytic effects on the peripheral trigeminal nerve. Saline and 90% ethanol were injected in control animals. One week after the injection, histopathological changes were noted in both glycerol and alcohol groups. In the former group, axonolysis and demyelination were restricted to the outer zone of the nerve bundles. Centrally located axons remained undamaged. A total destruction of all axons was found in the alcohol group. Four weeks after the injection in the glycerol group, small sized axons with thin myelin replaced damaged axons at the periphery of the bundle. No signs of regeneration were noted in the alcohol group. A possible mode of action of glycerol injected at the peripheral trigeminal nerve in relieving trigeminal neuralgia is described [41].

Myelin Sheath Repair by Fatty Acids

Eicosapentaenoic acid (EPA), a fatty acid present in high amount in fish, modulates immune response and stimulates myelin gene expression. In the present paper, we investigated the effects of EPA in an established animal model for multiple sclerosis (MS): experimental autoimmune encephalomyelitis (EAE) induced in dark agouti rats [42]. Diets supplemented either with 0.2% or 0.4% of EPA were administrated daily from the day of induction until the end of experiment. One group of rats received diet supplemented with 0.2% of EPA 10 days before induction. The control group (immunized rats) was fed with chow diet. The animals were analyzed at two different stages of the disease: during the acute phase (14 d.p.i.) and during the recovery phase (32 d.p.i.). We showed a delayed onset of clinical severity of disease in all groups of rats fed EPA-supplemented diets. This effect was associated to an increased expression of myelin proteins and an improved integrity of the myelin sheath as well as an up-regulation of FoxP3 expression in the central nervous system during the acute phase of EAE. No significant changes in T cell subsets were noted at the periphery. On the contrary, during the recovery phase of EAE, in animals assuming EPA-supplemented diet, an increase of CD4(+) CD25(+) and CD4(+) CD25(+) FoxP3(+) in peripheral lymphocytes was noted. Our results indicate that EPA-supplemented diets may provide benefits to MS patients [42].

Acute treatment of stroke with histone deacetylase (HDAC) inhibitors has been shown to reduce ischemic cell damage; however, it is unclear whether delayed treatment with HDAC inhibitors will contribute to the brain repair and plasticity. In the present study, we investigated the effects of delayed treatment of stroke with a pan HDAC inhibitor, valproic acid (VPA), on white matter injury and neurogenesis during stroke recovery [43]. Administration of VPA at a dose of 100mg/kg for 7 days starting 24h after middle cerebral artery occlusion (MCAo) in rats significantly improved neurological outcome measured 7-28 days post-MCAo. In addition, the VPA treatment significantly increased oligodendrocyte survival and newly generated oligodendrocytes, which was associated with elevation of myelinated axonal density in the ischemic boundary 28 days after MCAo. VPA treatment also increased the expression of glutamate transporter 1 (GLT1) in the ischemic boundary after stroke and increased acetylated histone H4 expression in neuroblasts and the number of new neurons in striatal ischemic boundary region. This study provides new evidence that the delayed VPA treatment enhances white matter repair and neurogenesis in ischemic brain, which may contribute to improved functional outcome [43].

Multiple sclerosis is a demyelinating disease with severe neurological symptoms due to blockage of signal conduction in affected axons. Spontaneous remyelination via endogenous progenitors is limited and eventually fails. Recent reports showed that forced expression of some transcription factors within the brain converted somatic cells to neural progenitors and neuroblasts. Here, we report the effect of valproic acid(VPA) along with forced expression of Oct4 transcription factor on lysolecithin (LPC)-induced experimental demyelination [44]. Mice were gavaged with VPA for one week, and then inducible Oct4 expressing lentiviral particles were injected into the lateral ventricle. After one-week induction of Oct4, LPC was injected into the optic chiasm. Functional remyelination was assessed by visual-evoked potential (VEP) recording. Myelination level was studied using Fluoro Myelin staining and immunohistofluorescent (IHF) against proteolipid protein (PLP). IHF was also performed to detect Oct4 and SSEA1 as pluripotency markers and Olig2, Sox10, CNPase and PDGFRα as oligodendrocyte lineage markers. One week after injection of Oct4 expressing vector, pluripotency markers SSEA1 and Oct4 were detected in the rims of the 3rd ventricle. LPC injection caused extensive demyelination and significantly delayed the latency of VEP wave. Animals pre-treated with VPA+Oct4 expressing vector, showed faster recovery in the VEP latency and enhanced myelination. Immunostaining against oligodendrocyte lineage markers

showed an increased number of Sox10+ and myelinating cells. Moreover, transdifferentiation of some Oct4-transfected cells (GFP+cells) to Olig2+ and CNPase+ cells were confirmed by immunostaining. One-week administration of VPA followed by one-week forced expression of Oct4 enhanced myelination by converting transduced cells to myelinating oligodendrocytes. This finding seems promising for enhancing myelin repair within the adult brains [44].

Oligodendrocytes have limited ability to repair the damage to themselves or to other nerve cells, as seen in demyelinating diseases like multiple sclerosis. An important strategy may be to replace the lost oligodendrocytes and/or promote the maturation of undifferentiated oligodendrocyte precursor cells (OPCs). Recent studies show that a composite of co-ultramicronized N-palmitoylethanolamine (PEA) and luteolin (co-ultramicronized PEA/luteolin, 10:1 by mass) is efficacious in improving outcome in experimental models of spinal cord and traumatic brain injuries. Here, we examined the ability of co-ultramicronized PEA/luteolin to promote progression of OPCs into a more differentiated phenotype. OPCs derived from newborn rat cortex were placed in culture and treated the following day with 10 μM co-ultramicronized PEA/luteolin [45]. Cells were collected 1, 4 and 8 days later and analyzed for expression of myelin basic protein (MBP). qPCR and Western blot analyses revealed a time-dependent increase in expression of both mRNA for MBP and MBP content, along with an increased expression of genes involved in lipid biogenesis. Ultramicronized PEA or luteolin, either singly or in simple combination, were ineffective. Further, co-ultramicronized PEA/luteolin promoted morphological development of OPCs and total protein content without affecting proliferation. Co-ultramicronized PEA/luteolin may represent a novel pharmacological strategy to promote OPC maturation [45].

Adult oligodendrocyte precursor cells (OPCs) are located adjacent to demyelinated lesion and contribute to myelin repair. The crucial step in remyelination is the migration of OPCs to the demyelinated area; however, the mechanism of OPC migration remains to be fully elucidated. Here we show that prostacyclin (prostaglandin I2, PGI2) promotes OPC migration, thereby promoting remyelination and functional recovery in mice after demyelination induced by injecting lysophosphatidylcholine (LPC) into the spinal cord [46]. Prostacyclin analogs enhanced OPC migration via a protein kinase A (PKA)-dependent mechanism, and prostacyclin synthase expression was increased in the spinal cord after LPC injection. Notably, pharmacological inhibition of prostacyclin receptor (IP receptor) impaired remyelination and motor recovery, whereas the administration of a prostacyclin analog promoted remyelination and motor recovery after LPC injection. Our results suggest that prostacyclin could be a key molecule for facilitating the migration of OPCs that are essential for repairing demyelinated areas, and it may be useful in treating disorders characterized by demyelination [46].

Dietary polyunsaturated fatty acids (PUFAs) have been postulated as alternative supportive treatment for multiple sclerosis, since they may promote myelin repair. We set out to study the effect of supplementation with n-3 and n-6 PUFAs on OLN-93 oligodendroglia and rat primary oligodendrocyte differentiation *in vitro* [47]. It appeared that OLN-93 cells actively incorporate and metabolise the supplemented PUFAs in their cell membrane. The effect of PUFAs on OLN-93 differentiation was further assessed by morphological and Western blot evaluation of markers of oligodendroglia differentiation: 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), zonula occludens-1 (ZO-1) and myelin-associated glycoprotein (MAG). Supplementation of the OLN-93 cells with n-3 and n-6 PUFAs increased the degree of differentiation determined by morphological analysis. Moreover, CNP protein expression was significantly increased by gamma-linolenic acid (GLA, 18:3n-6) supplementation. In accordance with the OLN-93 results, studies with rat primary oligodendrocytes, a more advanced model of cell differentiation, showed GLA supplementation to promote oligodendrocyte differentiation. Following GLA supplementation, increased numbers of proteolipid protein (PLP)-positive oligodendrocytes and increased myelin sheet formation was observed during differentiation of primary oligodendrocytes. Moreover, increased CNP, and enhanced PLP and myelin basic protein expression were found after GLA administration. These studies provide support for the dietary supplementation of specific PUFAs to support oligodendrocyte differentiation and function [47].

Demyelination is a pathological process characterized by the loss of myelin around axons. In the central nervous system, oligodendroglial damage and demyelination are common pathological features characterizing white matter and neurodegenerative disorders. Remyelination is a regenerative process by which myelin sheaths are restored to demyelinated axons, resolving functional deficits. This process is often deficient in demyelinating diseases such as multiple sclerosis (MS), and the reasons for the failure of repair mechanisms remain unclear. The characterization of these mechanisms and the factors involved in the proliferation, recruitment, and differentiation of oligodendroglial progenitor cells is key in designing strategies to improve remyelination in demyelinating disorders. First, a very dynamic combination of different molecules such as growth factors, cytokines, chemokines, and different signaling pathways is tightly regulated during the remyelination process. Second, factors unrelated to this pathology, i.e., age and genetic background, may impact disease progression either positively or negatively, and in particular, age-related remyelination failure has been proven to involve oligodendroglial cells aging and their intrinsic capacities among other factors. Third, nutrients may either help or hinder disease progression. Experimental evidence supports the anti-inflammatory role of omega-6 and omega-3 polyunsaturated fatty acids through the competitive inhibition of arachidonic acid, whose metabolites participate in inflammation, and the reduction in T cell proliferation. In turn, vitamin D intake and synthesis have been associated with lower MS incidence levels, while vitamin D-gene interactions might be involved in the pathogenesis of MS. Finally, dietary polyphenols have been reported to mitigate demyelination by modulating the immune response [48].

Peripheral nerve trauma induces the expression of genes presumed to be involved in the process of nerve degeneration and repair. In the present study, an *in vivo* paradigm was employed to identify molecules which may have important roles in these processes. A cDNA library was constructed with RNA extracted from rat dorsal root ganglia (DRG) 3 days after a sciatic nerve crush. After differential hybridization to this library, several cDNAs were identified that encoded mRNAs that were upregulated in the DRG ipsilateral to the crush injury, as opposed to the contralateral or naive DRG [49]. Approximately 0.15% of all the clones screened were found to be induced. This report presents the types of induced sequences identified and characterizes one of them, DA11. The 0.7 kb DA11 full length cDNA clone contains a 405-nucleotide open reading frame that encodes a putative protein of 15.2 kDa (135 amino acid residues) and is a member of the family of fatty acid binding proteins (FABP). The DA11 protein differs by one amino acid residue from the sequence of the C-FAPB protein and by eight residues from the sequence of mal1, proteins found in rat and mouse skin, respectively. Northern and Western blot analyses showed that the DA11 mRNA and protein were induced in the injured DRG. Furthermore, studies using antibodies generated against DA11 found that the DA11-like immunoreactivity was more pronounced in the nuclei of neurons located in the DRG ipsilateral to the sciatic cut than those located in the contralateral DRG. The induction of DA11 mRNA and protein in DRG neurons suggests, for the first time, the involvement of a neuronal FABP in the process of degeneration and repair in the nervous system [49].

To study the effect of the loaded concentration gradient of nerve growth factor (NGF) immobilized conduit on rat peripheral nerve defect repair [50]. The peripheral nerve conduits made of poly (epsilon-caprolactone)-block-poly (L-lactide-co-epsilon-caprolactone) were prepared with uniform loads or concentration gradient loads by combining differential absorption of NGF/silk fibroin (SF) coating, and the gradient of NGF was immobilized in the nerve conduits. ELISA method was used to exam the NGF release for 12 weeks *in vitro*. Twenty-four male Sprague Dawley rats (weighing, 220-250 g) were selected to establish the right sciatic nerve defect model (14 mm in length) and randomly divided into 4 groups according to repair methods. The transected nerve was bridged by a blank conduit without NGF in group A, by a conduit containing uniform loads of NGF in group B, by a conduit concentration gradient loads of NGF in group C, and by the autogenous nerve segment in group D. The gross observation, electrophysiological examination, histological observation, and transmission electron microscope observation were carried out to assess the nerve regeneration at 12 weeks after surgery.

The cumulative release amount of NGF was (14.2 +/- 1.4) ng/mg and (13.7 +/- 1.3) ng/mg in gradient of NGF loaded conduits and uniform NGF loaded conduits respectively at 12 weeks, showing no significant difference ($t=0.564$, $P=0.570$). All the animals survived to completion of the experiment; plantar ulcers occurred at 4 days, which healed at 12 weeks; groups C and D were better than groups A and B in ulcerative healing. At 12 weeks after surgery, the compound muscle action potential of group A was significantly lower than that of groups B, C, and D ($P<0.05$), and group B was significantly lower than groups C and D ($P<0.05$), but no significant difference was found between groups C and D ($P>0.05$). The axon density of group C was significantly higher that of groups A, B, and D ($P<0.05$); group D was significantly higher than groups A, B, and C, and group C was significantly higher than groups A and B in the axon number, axon diameter, and area of muscle fiber ($P<0.05$); the thickness of myelin sheath of groups C and D was significantly larger than that of groups A and B ($P<0.05$), but no significant difference was found between groups C and D ($P>0.05$).

Gradient of NGF loaded nerve condnits for rat sciatic nerve defect has similar results to autogenous nerve, with a good bridge, which can promote the sciatic nerve regeneration, improve the myelinization of the regenerating nerve, and accelerate the function reconstruction of the regenerating nerve [50].

Our previous work showed an early development of behavioral reflexes in rats whose mothers had been fed, during pregnancy and lactation, a lipid fraction extracted from yeast grown on n-alkanes (which contain 50% odd-chain fatty acids) in comparison with controls fed a margarine diet [51]. To clarify whether the observed changes might be linked to an early myelination, we have investigated mRNAs involved in myelin synthesis in the brains of offspring at 5 days of age by northern blot and in situ hybridization. Northern blot analysis showed that proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) mRNAs were higher in animals on the lipid diet compared with controls. In-situ hybridization with probes specific for PLP, myelin basic protein, and MOG mRNA showed significantly higher numbers of positive cells in test animals compared with controls in all brain regions. This study shows an acceleration of myelinogenesis induced by dietary lipids. These data can give a new insight in the therapeutical approaches involved to promote repair in demyelinating diseases [51].

White matter injury induced by ischemic stroke elicits sensorimotor impairments, which can be further deteriorated by persistent proinflammatory responses. We previously reported that delayed and repeated treatments with omega-3 polyunsaturated fatty acids (n-3 PUFAs) improve spatial cognitive functions and hippocampal integrity after ischemic stroke [52]. In the present study, we report a post-stroke n-3 PUFA therapeutic regimen that not only confers protection against neuronal loss in the gray matter but also promotes white matter integrity. Beginning 2h after 60 min of middle cerebral artery occlusion (MCAO), mice were randomly assigned to receive intraperitoneal docosahexaenoic acid (DHA) injections (10 mg/kg, daily for 14 days), alone or in combination with dietary fish oil (FO) supplements starting 5 days after MCAO. Sensorimotor functions, gray and white matter injury, and microglial responses were examined up to 28 days after MCAO. Our results showed that DHA and FO combined treatment-facilitated long-term sensorimotor recovery and demonstrated greater beneficial effect than DHA injections alone. Mechanistically, n-3 PUFAs not only

offered direct protection on white matter components, such as oligodendrocytes, but also potentiated microglial M2 polarization, which may be important for white matter repair. Notably, the improved white matter integrity and increased M2 microglia were strongly linked to the mitigation of sensorimotor deficits after stroke upon n-3 PUFA treatments. Together, our results suggest that post-stroke DHA injections in combination with FO dietary supplement benefit white matter restoration and microglial responses, thereby dictating long-term functional improvements [52].

Attempts have been made to improve nerve conduits in peripheral nerve reconstruction. We investigated the potential therapeutic effect of adipose-derived mesenchymal cells (ASCs) and ghrelin (GHR), a neuropeptide with neuroprotective, trophic, and developmental regulatory actions, on peripheral nerve regeneration in a model of severe nerve injury repaired with nerve conduits [53]. The right sciatic nerves of 24 male Wistar rats were 10-mm transected unilaterally and repaired with Dl-lactic-ε-caprolactone conduits. Rats were then treated locally with saline, ASCs, or GHR. At 12 weeks post-surgery, we assessed limb function by measuring ankle stance angle and percentage muscle mass reduction and evaluated the histopathology, immunohistochemistry, ultrastructure, and morphometry of myelinated fibers.

Rats receiving GHR or ASCs showed no significant increased functional recovery in ankle stance angle ($p=0.372$) but a higher nerve area ($p=0.015$), myelin area ($p=0.046$) and number of myelinated fibers ($p=0.012$) in the middle and distal segments of operated sciatic nerves in comparison to saline-treated control animals. These results suggest that utilization of ghrelin or ASCs may improve nerve regeneration using Dl-lactic-ε-caprolactone conduits [53].

A technique is described for producing large demyelinating lesions of the spinal cord in the guinea pig. Guinea pigs were pre-treated by immunization with ovalbumin and water-soluble adjuvant (N-acetyl-muramyl L-alanyl D-isoglutamine, MDP) in water-in-oil emulsion (Freund's incomplete adjuvant). They were given a large dose (10 mg) of ovalbumin i.p. one month later. After a few weeks the animals were sensitized with guinea-pig basic protein in Freund's complete adjuvant. Five out of 11 animals developed large, distinctive, sharply demarcated, symmetrical demyelinating lesions within 30 days. These lesions occurred in the dorsal and anterior columns, root entry zones and subpial region of the spinal cord. Histology showed a considerable amount of free lipids. There were also infiltrative lesions of classical experimental allergic encephalomyelitis (EAE) of normal severity in the same animals. The demyelinating lesions resembled those seen in multiple sclerosis in their location and extent in the spinal cord and in the presence of free lipids. Control experiments indicated that pretreatment with ovalbumin/MDP and the second injection of ovalbumin was necessary for all the demyelination; moreover, guinea pigs immunized with basic protein in Freund's complete adjuvant or Freund's incomplete adjuvant plus MDP without pretreatment only developed classical EAE with minimal or no demyelination [54].

The major portion of the endoneural lipids is found in myelin. Since perineurial cells differ morphologically from endoneural cell components, we attempted to determine whether these morphological differences also extended to a difference in fatty acid (FA) composition. Under normal circumstances, unsaturated FAs are more abundant than saturated ones (55-60% of total FAs) in endoneurium and perineurium [55]. A characteristic biochemical difference between these two structures lies in the distribution of linoleic acid (C18:2(n-6)) which represents 20% of total FAs in perineurium and only 2% in endoneurium. Wallerian degeneration takes place after injection of pure glycerol into the endoneurium. This is followed by regeneration characterized by a proliferation of perineurial cells infiltrating the center of the nerve fascicule forming microcompartments. The changes in linoleic acid content reflect these morphological changes. A marked increase in linoleic acid is detected in the endoneurial fraction in parallel with the observed infiltration of perineurial cells into the nerve fascicule [55].

White matter (WM) abnormalities have been implicated in schizophrenia, yet the mechanisms underlying these abnormalities are not fully understood. Several lines of evidence suggest that polyunsaturated fatty acids (PUFAs) play a role in myelination, and there is substantial evidence documenting decreased PUFA concentrations in schizophrenia. We therefore hypothesized that lower membrane PUFA concentrations may be related to reduced WM integrity in schizophrenia and related disorders.

In 30 male patients with a recent-onset psychotic disorder, erythrocyte membrane PUFA concentrations were assessed and diffusion tensor imaging was performed with voxel wise analysis.

Lower total PUFA concentration was associated with lower fractional anisotropy (FA) throughout the corpus callosum and bilateral parietal, occipital, temporal and frontal WM ($P<0.05$, corrected). Of the individual PUFAs, lower arachidonic acid concentration, and to a lesser extent, lower nervonic acid, linoleic acid, and docosapentaenoic acid concentration were significantly associated with lower FA. PUFA concentrations were inversely associated with radial diffusivity but showed little association with axial diffusivity. Greater severity of negative symptoms was associated with lower nervonic acid concentration and lower FA values.

Membrane PUFA concentrations appear to be robustly related to brain WM integrity in early phase psychosis. These findings may provide a basis for studies to investigate the effects of PUFA supplementation on WM integrity and associated symptomatology in early psychosis [56]. Diabetic polyneuropathy is a serious complication of diabetes mellitus and the most frequent neuropathy worldwide. This study was designed to investigate the possible beneficial effects of evening primrose oil (EPO) on histopathological changes of sciatic nerves in streptozotocin-induced diabetic rats [57].

The rats were randomly allotted into three experimental groups: A (control), B (diabetic untreated), and C (diabetic treated with EPO); each group contained 10 animals. Groups B and C received streptozotocin (STZ) to induce diabetes.

The rats in group C were given EPO for 2 weeks after 6 weeks of STZ injection. Blood and tissue samples were obtained for biochemical and histopathological investigation.

STZ-treated diabetic rats showed reduction of the size of islets of Langerhans, fatty degeneration in the pancreatic acini with dilation, irregularity, and increased thickness of blood vessels. Electron micrography of sciatic nerves of diabetic rats showed multiple vaculations and partial separation of myelinated nerve fibers with axonal atrophy, endoneural edema, and increased collagen fibers. Compared with diabetic rats, EPO induced partial recovery from diabetes-induced pancreatic and nerve damage.

Histologic evaluation of the tissues in diabetic animals treated with EPO showed fewer morphologic alterations with significant decrease of myelin breakdown. Furthermore, the ultrastructural features of axons showed partial improvement. It is believed that further preclinical research into the utility of EPO may indicate its usefulness as a potential treatment on peripheral neuropathy in STZ-induced diabetic rats [57].

Polyunsaturated fatty acids are known to affect the immune response and administration of the omega-6 fatty acid linoleic acid has been reported to be beneficial in multiple sclerosis (MS) and EAE. In this study we have investigated the effects of oral feeding of plant lipid rich in the omega-6 fatty acid gamma-linolenic acid from Borago officinalis on acute and relapse disease and the immune response in EAE using SJL mice [58]. EAE was induced by an encephalitogenic peptide (92-106) of myelin oligodendrocyte glycoprotein (MOG), and mice were fed the plant lipid daily from 7 days after EAE induction to assess the effects on acute disease and from day 25 to assess the effects on disease relapse. The clinical incidence and histological manifestations of acute EAE, and the clinical relapse phase of chronic relapsing EAE (CREAE) were markedly inhibited by omega-6 fatty acid feeding. A significant increase in the production of TGF-beta1 in response to concanavalin A (Con A) at day 13 and a significant increase in TGF-beta1 and PGE2 to Con A, PPD and MOG peptide (92-106) at day 21 were detected in spleen mononuclear cells from fatty acid-fed mice. There was no difference in interferon-gamma, IL-4 and IL-2 production between the fatty acid-fed and control groups. Significantly higher TGF-beta mRNA expression was found in the spleens of omega-6 fatty acid-fed mice at day 21. There were no differences in spleen cell proliferative response to Con A, PPD and MOG peptide (92-106). Biochemical analysis of spleen cell membrane fatty acids revealed significant increases in the eicosanoid precursor fatty acids dihomo-gamma-linolenic acid and arachidonic acid in response to gamma-linolenic acid feeding, indicating rapid metabolism to longer chain omega-6 fatty acids. These results show that oral feeding of gamma-linolenic acid-rich plant lipid markedly affects the disease course of acute EAE and CREAE and is associated with an increase in cell membrane long chain omega-6 fatty acids, production of PGE2 and gene transcription and, on activation, secretion of TGF-beta1 [58].

Expression of brain fatty acid-binding protein (B-FABP) is spatially and temporally correlated with neuronal differentiation during brain development. Isothermal titration calorimetry demonstrates that recombinant human B-FABP clearly exhibits high affinity for the polyunsaturated n-3 fatty acids alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and for monounsaturated n-9 oleic acid(K(d) from 28 to 53 nm) over polyunsaturated n-6 fatty acids, linoleic acid, and arachidonic acid (K(d) from 115 to 206 nm). B-FABP has low binding affinity for saturated long chain fatty acids. The three-dimensional structure of recombinant human B-FABP in complex with oleic acid shows that the oleic acid hydrocarbon tail assumes a "U-shaped" conformation, whereas in the complex with docosahexaenoic acid the hydrocarbon tail adopts a helical conformation. A comparison of the three-dimensional structures and binding properties of human B-FABP with other homologous FABPs, indicates that the binding specificity is in part the result of nonconserved amino acid Phe(104), which interacts with double bonds present in the lipid hydrocarbon tail. In this context, analysis of the primary and tertiary structures of human B-FABP provides a rationale for its high affinity and specificity for polyunsaturated fatty acids. The expression of B-FABP in glial cells and its high affinity for docosahexaenoic acid, which is known to be an important component of neuronal membranes, points toward a role for B-FABP in supplying brain abundant fatty acids to the developing neuron [59].

We have previously shown that a mixture of dietary conjugated derivatives of linoleic acid (conjugated linoleic acid, CLA) induces peroxisome proliferator-responsive enzymes and modulates hepatic lipid metabolism *in vivo* [60]. The present studies demonstrate that CLA is a high affinity ligand and activator of peroxisome proliferator-activated receptor alpha (PPARalpha) and induces accumulation of PPAR-responsive mRNAs in a rat hepatoma cell line. Using a scintillation proximity assay (SPA), CLA isomers were shown to be ligands for human PPARalpha with a rank order of potency of (9Z,11E)>(10E,12Z)>(9E,11E)> furan-CLA (IC (50) values from 140nm to 400nm). Levels of acyl-CoA oxidase (ACO), liver fatty acid-binding protein (L-FABP), and cytochrome P450IVA1 (CYP4A1) mRNA were induced by CLA in FaO hepatoma cells. Even though linoleate and CLA were incorporated into lipids of hepatoma cells to the same extent, linoleate had little or no effect on ACO, CYP4A1, or L-FABP mRNA. In agreement with its binding potency, (9Z,11E)-CLA was the most efficacious PPARalpha activator in the mouse PPARalpha-GAL4(UAS) (5)-CAT reporter system. These data indicate that CLA is a ligand and activator of PPARalpha and its effects on lipid metabolism may be attributed to transcriptional events associated with this nuclear receptor. Also, (9Z,11E)-CLA is one of the most avid fatty acids yet described as a PPARalpha ligand [60].

Adipocytes express two lipid-binding proteins; the major one termed the adipocyte lipid-binding protein or aP2 (ALBP/aP2) and a minor one referred to as the keratinocyte lipid-binding protein (KLBP). In order to evaluate the potential physiological roles for these proteins, their biochemical and biophysical properties have been analyzed and compared. ALBP/aP2 and KLBP exhibit similar binding affinities for most long-chain fatty acids; however, ALBP/aP2 exhibits a

two to three-fold increased affinity for myristic, palmitic, oleic and linoleic acids, the predominant fatty acids of adipocytes. As measured by guanidinium hydrochloride denaturation, the stability of ALBP/aP2 is nearly 3 kcal/mol greater than that of KLBP. While the pI of ALBP/aP2 was determined to be 9.0, that of KLBP is 6.5 suggesting differing net charges at physiological pH. Analysis of surface electrostatic properties of ALBP/aP2 and KLBP revealed similar charge polarity, although differences in the detailed charge distribution exist between the proteins. The distribution of hydrophobic patches was also different between the proteins, ALBP/aP2 has only scattered hydrophobic surfaces while KLBP has a large hydrophobic patch near the ligand portal into the binding cavity. In sum, these results point out that despite the striking similarity between ALBP/aP2 and KLBP in tertiary structure, significant differences in ligand binding and surface properties exist between the two proteins. Hence, while it is tempting to speculate that ALBP/aP2 and KLBP are metabolically interchangeable, careful analysis suggests that the two proteins are quite distinct and likely to play unique metabolic roles [61].

A fatty acid-binding protein from the nematode Ascaridia galli was characterized. The gene was isolated and recombinantly expressed in Escherichia coli. According to the deduced amino acid sequence A. galli fatty acid-binding protein (AgFABP) belongs to the family of nematode polyprotein allergens, as shown by Western blotting and PCR analysis with genomic DNA and cDNA. Both native and recombinant proteins bind fatty acids and retinoids with high affinity. The fluorescent fatty acid analogue 11-[(5-dimethylaminonaphthalene-1-sulfonyl)amino] undecanoic acid (DAUDA) shows substantial changes in its emission spectrum when bound to AgFABP; this binding is reversed by fatty acids such as oleate. Moreover, changes of the intrinsic fluorescence of retinol and retinoic acid confirm retinoid binding activity of AgFABP. Fluorescence titration experiments with DAUDA indicate stoichiometric binding to a single binding site per monomer unit with affinities (Kd) of 1.6 and 1.8x10(-7) m for native and the recombinant protein, respectively. The apparent binding affinities of the nonfluorescent ligands were calculated in displacement experiments with DAUDA and values in the same range were obtained for myristic, palmitic, oleic, linoleic, arachidonic and retinoic acid. Additionally, the binding affinity of AgFABP for oleate and palmitate was determined by direct and indirect radiochemical analysis and the values obtained were similar to those from the fluorescent experiments. Both proteins show a preference for the binding of long-chain saturated and unsaturated fatty acids, but not for short chain (C3-C12) and branched fatty acids, cholesterol and tryptophan [62].

To determine the transport and utilization of dietary saturated, monounsaturated, and n-6 and n-3 polyunsaturated fatty acids for the developing brain and other organs, artificially reared rat pups were fed a rat milk substitute containing the perdeuterated (each 97 atom% deuterium) fatty acids, i.e., palmitic, stearic, oleic, linoleic, and linolenic, from day 7 after birth to day 14 as previously described. Fatty acids in lipid extracts of the liver, lung, kidney, and brain were analyzed by gas chromatography-mass spectrometry to determine their content of each of the deuterated fatty acids. The uptake and metabolism of perdeuterated fatty acid lead to the appearance of three distinct groups of isotopomers: the intact perdeuterated, the newly synthesized (with recycled deuterium), and the natural unlabelled fatty acid. The quantification of these isotopomers permits the estimation of uptake and de novo synthesis of these fatty acids. Intact perdeuterated palmitic, stearic, and oleic acids from the diet were found in liver, lung, and kidney, but not in brain. By contrast, perdeuterated linoleic acid was found in all these organs. Isotopomers of fatty acid from de novo synthesis were observed in palmitic, oleic, and stearic acids in all tissues. The highest enrichment of isotopomers with recycled deuterium was found in the brain. The data indicate that, during the brain growth spurt and the prelude to myelination, the major saturated and monounsaturated fatty acids in brain lipids are exclusively produced locally by de novo biosynthesis. Consequently, the n-6 and n-3 polyunsaturated fatty acids must be transported and delivered to the brain by highly specific mechanisms [63].

The endoneurial nonpolar lipids were serially examined throughout Wallerian degeneration and regeneration. Following nerve crush in the rat, the endoneurial content of cholesterol falls and cholesteryl ester content rises dramatically. The maximal alteration of this ratio corresponds reasonably well with events of myelin ovoid dissolution to sudanophilic amorphous lipids. Thereafter, as regenerative events overshadow degenerative events the ratio is slowly restored toward normal. The increased cholesteryl esters are probably synthesized within endoneurium from free fatty acids which become available when myelin is degraded. The endoneurial free fatty acid content presumably represents the net effect of phospholipid degradation, cholesterol esterification, cholesteryl ester hydrolysis, and fascicular entry and exit. Free fatty acids become significantly elevated by 12 days, probably reach a peak between 16 and 60 days, and thereafter return to normal with fiber regeneration. The fatty acid composition of cholesteryl esters from crushed nerves is markedly different from those of normal sciatic nerves. The altered fatty acid composition of cholesteryl esters from myelin suggests that both synthesis and hydrolysis exhibit substrate specificity toward chain length and unsaturation, with oleate being the most favoured substrate [64].

Lipid Emulsion Effects on Mitochondria and Intracellular Calcium

Local anesthetic toxicity is thought to be mediated partly by inhibition of cardiac mitochondrial function. Intravenous (i.v.) lipid emulsion may overcome this energy depletion, but doses larger than currently recommended may be needed for rescue effect. In this randomized study with anesthetized pigs, we compared the effect of a large dose, 4 mL/kg, of i.v. 20% Intralipid® (n=7) with Ringer's acetate (n=6) on cardiovascular recovery after a cardiotoxic dose of bupivacaine [18]. We also examined mitochondrial respiratory function in myocardial cell homogenates analyzed promptly after needle biopsies from the animals.

Bupivacaine plasma concentrations were quantified from plasma samples. Arterial blood pressure recovered faster, and systemic vascular resistance rose more rapidly after Intralipid than Ringer's acetate administration ($p<0.0001$), but Intralipid did not increase cardiac index or left ventricular ejection fraction. The lipid-based mitochondrial respiration was stimulated by approximately 30% after Intralipid ($p<0.05$) but unaffected by Ringer's acetate. The mean (standard deviation) area under the concentration-time curve (AUC) of total bupivacaine was greater after Intralipid (105.2(13.6)mg·min/L) than after Ringer's acetate (88.1(7.1)mg·min/L) ($p=0.019$). After Intralipid, the AUC of the lipid-un-entrapped bupivacaine portion (97.0(14.5) mg·min/L) was 8% lower than that of total bupivacaine ($p<0.0001$). To conclude, 4 mL/kg of Intralipid expedited cardiovascular recovery from bupivacaine cardiotoxicity mainly by increasing systemic vascular resistance. The increased myocardial mitochondrial respiration and bupivacaine entrapment after Intralipid did not improve cardiac function [65].

Lipid emulsions have been used to treat various drug toxicities and for total parenteral nutrition therapy. Their usefulness has also been confirmed in patients with local anesthetic-induced cardiac toxicity. The purpose of this study was to measure the hemodynamic and composition effects of lipid emulsions and to elucidate the mechanism associated with changes in intracellular calcium levels in myocardiocytes.

We measured hemodynamic effects using a digital analysis system after Intralipid® and Lipofundin® MCT/LCT were infused into hearts hanging in a Langendorff perfusion system [20]. We measured the effects of the lipid emulsions on intracellular calcium levels in H9c2 cells by confocal microscopy.

Infusion of Lipofundin® MCT/LCT 20% (1ml/kg) resulted in a significant increase in left ventricular systolic pressure compared to that after infusing modified Krebs-Henseleit solution (1ml/kg) ($P=0.003$, 95% confidence interval [CI], 2.4-12.5). Lipofundin® MCT/LCT 20% had a more positive inotropic effect than that of Intralipid® 20% ($P = 0.009$, 95% CI, 1.4-11.6). Both lipid emulsion treatments increased intracellular calcium levels. Lipofundin® MCT/LCT (0.01%) increased intracellular calcium level more than that of 0.01% Intralipid® ($P<0.05$, 95% CI, 0.0-1.9).

These two lipid emulsions had different inotropic effects depending on their triglyceride component. The inotropic effect of lipid emulsions could be related with intracellular calcium level [66].

Accidental intravascular or high-dose injection of local anesthetics (LA) can result in serious, potentially life-threatening complications. Indeed, adequate supportive measures and the administration of lipid emulsions are required in such complications. The study's objectives were threefold: (i) evaluate the myocardial toxicity of levobupivacaine when administered intravenously; (ii) investigate levobupivacaine toxicity on cardiomyocytes mitochondrial functions and cellular structure; (iii) assess the protective effects of a lipid emulsion in the presence or absence of myocardial ischemia. Domestic pigs randomized into two groups of 24 animals each, with either preserved coronary circulation or experimental myocardial ischemia. Six animals from each group received either: (i) single IV injection of saline, (ii) lipid emulsion (Intralipid(®)), (iii) levobupivacaine, (iv) combination levobupivacaine-Intralipid(®) . Serially measured endpoints included: heart rate, duration of the monophasic action potentials (dMAP), mean arterial pressure, and peak of the time derivative of left ventricular pressure (LV dP/dtmax). In addition, the following cardiomyocytes mitochondrial functions were measured: reactive oxygen species (ROS) production, oxidative phosphorylation, and calcium retention capacity (CRC) as well as the consequences of ROS production on lipids, proteins, and DNA. IV injection of levobupivacaine induced sinus bradycardia and reduced dMAP and LV dP/dtmax. At the mitochondrial level, oxygen consumption and CRC were decreased. In contrast, ROS production was increased leading to enhanced lipid peroxidation and structural alterations of proteins and DNA. Myocardial ischemia was associated with global worsening of all changes. Intralipid(®) quickly improved haemodynamics. However, beneficial effects of Intralipid(®) were less clear after myocardial ischemia [67].

Cocaine intoxication leads to over 500,000 emergency department visits annually in the United States and ethanol cointoxication occurs in 34% of those cases. Cardiotoxicity is an ominous complication of cocaine and cocaethylene overdose for which no specific antidote exists. Because infusion of lipid emulsion (Intralipid) can treat lipophilic local anesthetic toxicity and cocaine is an amphipathic local anesthetic, the authors tested whether lipid emulsion could attenuate cocaine cardiotoxicity *in vivo* [68]. The effects of lipid emulsion were compared with the metabolically inert sulfobutylether-β-cyclodextrin (SBE-β-CD; Captisol) in an isolated heart model of cocaine and cocaethylene toxicity to determine if capture alone could exert similar benefit as lipid emulsion, which exhibits multimodal effects. The authors then tested if cocaine and cocaethylene, like bupivacaine, inhibit lipid-based metabolism in isolated cardiac mitochondria.

For whole animal experiments, Sprague-Dawley rats were anesthetized, instrumented, and pretreated with lipid emulsion followed by a continuous infusion of cocaine to assess time of onset of cocaine toxicity. For *ex vivo* experiments, rat hearts were placed onto a nonrecirculating Langendorff system perfused with Krebs-Henseleit solution. Heart rate, left ventricle maximum developed pressure (LVdevP), left ventricle diastolic pressure, maximum rate of contraction (+dP/dtmax), maximum rate of relaxation (-dP/dtmax), rate-pressure product (RPP=heart rate×LVdevP), and line pressure were monitored continuously during the experiment. A dose response to cocaine (10, 30, 50, and 100 μmol/L) and cocaethylene (10, 30, and 50 μmol/L) was generated in the absence or presence of either 0.25% lipid emulsion or SBE-β-CD. Substrate-specific rates of oxygen consumption were measured in interfibrillar cardiac mitochondria in the presence of cocaine, cocaethylene, ecgonine, and benzoylecgonine.

Treatment with lipid emulsion delayed onset of hypotension (140 seconds vs. 279 seconds; p=0.008) and asystole (369 seconds vs. 607 seconds; p=0.02) in whole animals. Cocaine and cocaethylene induced dose-dependent decreases in RPP, +dP/dtmax, and -dP/dtmax abs (p<0.0001) in Langendorff hearts; line pressure was increased by cocaine and cocaethylene infusion, but not altered by treatment. Lipid emulsion attenuated cocaine- and cocaethylene-induced cardiac depression. SBE-β-CD alone evoked a mild cardio depressant effect (p<0.0001) but attenuated further cocaine- and cocaethylene-induced decrements in cardiac contractility at high concentrations of drug (100 μmol/L; p<0.001). Finally, both cocaine and cocaethylene, but not ecgonine and benzoylecgonine, inhibited lipid-dependent mitochondrial respiration by blocking carnitine exchange (p<0.05).

A commercially available lipid emulsion was able to delay progression of cocaine cardiac toxicity *in vivo*. Further, it improved acute cocaine- and cocaethylene-induced cardiac toxicity in rat isolated heart while SBE-β-CD was effective only at the highest cocaine concentration. Further, both cocaine and cocaethylene inhibited lipid-dependent mitochondrial respiration. Collectively, this suggests that scavenging-independent effects of lipid emulsion may contribute to reversal of acute cocaine and cocaethylene cardiotoxicity, and the beneficial effects may involve mitochondrial lipid processing [68].

We hypothesized that acute lipid-induced insulin resistance would be attenuated in high-oxidative muscle of lean trained (LT) endurance athletes due to their enhanced metabolic flexibility and mitochondrial capacity [69]. Lean sedentary (LS), obese sedentary (OS), and LT participants completed two hyperinsulinemic euglycemic clamp studies with and without (glycerol control) the coinfusion of Intralipid. Metabolic flexibility was measured by indirect calorimetry as the oxidation of fatty acids and glucose during fasted and insulin-stimulated conditions, the latter with and without lipid oversupply. Muscle biopsies were obtained for mitochondrial and insulin-signaling studies. During hyperinsulinemia without lipid, glucose infusion rate (GIR) was lowest in OS due to lower rates of nonoxidative glucose disposal (NOGD), whereas state 4 respiration was increased in all groups. Lipid infusion reduced GIR similarly in all subjects and reduced state 4 respiration. However, in LT subjects, fat oxidation was higher with lipid oversupply, and although glucose oxidation was reduced, NOGD was better preserved compared with LS and OS subjects. Mitochondrial performance was positively associated with better NOGD and insulin sensitivity in both conditions. We conclude that enhanced mitochondrial performance with exercise is related to better metabolic flexibility and insulin sensitivity in response to lipid overload [69].

Conclusion

Multiple sclerosis is a demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged. Trigeminal neuralgia involves loss of the myelin around the trigeminal nerve. Myelin is a phospholipid membrane that wraps around axons to provide them with insulation. It is produced by Schwann cells in the PNS, and by oligodendrocytes in the CNS. Intralipid treatment is first suggested in the medical literature as a way for myelin sheath repair in multiple sclerosis and trigeminal neuralgia.

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