Diabetic neuropathy (DN) is the most common complication of diabetes mellitus, and it imposes a considerable burden on a patient's quality of life and the health-care system. Despite the prevalence and severity of DN, there are no effective treatments.

This presentation will focus on Cutting-Edge Theories of Pathogenesis of Diabetic Neuropathy. One unifying model of injury lies in the ability of high glucose to both enhance production as well as decrease scavenging of reactive oxygen species, leading to cellular oxidative stress and impaired mitochondrial function. Excess glucose leads to an oversupply of electrons in the mitochondrial transfer chain that results in mitochondrial membrane hyperpolarization and mitochondrial overproduction of superoxide (1; 2), PARP activation (3) and decreased GAPDH activity (4). Decreased GAPDH activity activates the polyol pathway, increases intracellular AGE formation (5), activates PKC and subsequently NFkB, and activates hexosamine pathway flux (4; 6; 7). We will also review novel mechanisms of mitochondrial degeneration in the pathogenesis of DN, including interactions with oxidative stress, DNA damage, deregulation of mitochondrial fission and fusion proteins highlighting potential mitochondrial sites for therapeutic intervention (8).

Reference:

Il diabete mellito tipo 1 (T1DM), è una malattia autoimmune organo-specifica risultante dalla progressiva distruzione delle β-cellule insulinosecernenti delle isole di Langerhans del pancreas da parte di linfociti autoreattivi e loro mediatori. L'esordio clinico della malattia si può manifestare a tutte le età ed è preceduto da una fase preclinica che può durare da pochi mesi a vari anni, durante la quale le cellule insulinosecernenti vengono progressivamente distrutte. Nella storia naturale della malattia si distingue una fase iniziale caratterizzata dalla suscettibilità genetica sulla quale agisce un evento precipitante con conseguente perdita della tolleranza verso gli autoantigeni insulari. Inizia quindi un periodo preclinico di durata variabile, durante il quale il processo autoimmunitario di distruzione -cellulare è già iniziato, come è dimostrato dalla comparsa di autoanticorpi e di linfociti T autoreattivi diretti contro autoantigeni insulari (soprattutto insulinina, glutamminico-decarbossilasi e tirosina fosfatasi). Nella fase preclinica è, quindi, possibile identificare con notevole accuratezza ed attendibilità, attraverso il dosaggio di autoanticorpi specifici, quei soggetti che, pur non essendo ancora diabetici, stanno subendo un'aggressione autoimmune a danno delle proprie isole pancreatiche. Con il progredire della distruzione autoimmune e con la conseguente diminuzione del numero delle β-cellule funzionanti, iniziano a comparire le prime anomalie metaboliche: esse consistono nella mancanza della pulsatilità della secrezione insulinica e nella perdita del picco precoce di secrezione d'insulina in seguito a un carico endovenoso di glucosio. Infine una volta che la gran parte delle β-cellule è stata distrutta si assiste al vero e proprio esordio clinico della malattia, con il suo quadro clinico e metabolico. Il diabete autoimmune è quindi una malattia multifattoriale e poligenica; i fattori patogenetici principali, interagenti tra loro sono genetici, ambientali ed immunitari.
INFLAMMATION IN DIABETIC NEUROPATHIES

Uncini Antonino

Osp. SS Annunziata, Clinica Neurologica - Chieti

Diabetic neuropathy is the most common neuropathy in industrialized countries. Considering that diabetes affects an estimated 177 million people worldwide (World Health Organization (WHO), 2002), more than 20 million people suffer from diabetic neuropathy with a remarkable range of clinical manifestations. More than eight percent of the patients have a predominantly sensory distal symmetrical form that progress following a fibre-length dependent pattern. Other diabetic patients may develop focal and multifocal neuropathy that include cranial nerve involvement, limb and truncal neuropathies, proximal diabetic neuropathy of the lower limbs. Besides the clinical pattern of the neurological deficit, a major difference exists between the common length-dependent diabetic polyneuropathy (LDDP) and focal or multifocal neuropathies that occur in diabetic patients. The LDDP does not show any trend to improve and either relentlessly progresses or remain relatively stable over years. Conversely, the focal diabetic neuropathies may relapse but their course remain self limited.

There is increasing evidence that focal and multifocal diabetic neuropathies are associated with vascular and/or neural inflammation and the occurrence of a chronic inflammatory demyelinating neuropathy is 11 times higher among diabetic than non diabetic patients.

In this talk I will review the clinical and pathological aspects of diabetic neuropathies associated with inflammatory phenomena.
Peripheral neuropathy is one of the most common and debilitating long-term complications of type 1 and type 2 diabetes mellitus. Experimental diabetic neuropathy has a number of features in common with the human disease. Axonal degeneration and slowing in nerve conduction velocity (NCV) reflect loss of myelinated fibres and paranodal demyelination. Decreases in Na⁺,K⁺-ATPase activity in peripheral nerves and reduced intra-epidermal nerve fibre density linked with impaired nociceptive threshold have all been described in humans and streptozotocin (STZ)-induced neuropathy. Animal models of human diseases affecting the peripheral nervous system are widely used to investigate the pathogenesis of neurotoxicity and compare the effects of potential new therapeutic agents. Since the discovery of the neurotrophic and neuroprotective action of erythropoietin (EPO), we have demonstrated its efficacy in preventing and reversing nerve dysfunction in STZ-induced diabetes in rats. We overcame the potential drawback of EPO, namely the increase of red-cell mass caused by chronic treatment by employing a carbamylated EPO (CEPO) which maintains the neuroprotective efficacy of EPO, with comparable potency, but does not interact with the hematopoietic system. Future development should see peptides based on the biologically active portion of EPO. A new possibility might be neuroactive steroids. Neuroactive steroids, such as progesterone and testosterone and their derivatives have neuroprotective effects against STZ-induced diabetic neuropathy at the functional, biochemical and neuropathological levels. In addition, a group of peptides including neurotrophic factors, C-peptide, an islet neogenesis-associated protein peptide and prosaposin-derived peptide, are attracting interest for their potential in the treatment of diabetes-associated neuropathies and possibly in other neurodegenerative conditions. We will discuss some experimental models and new strategies in the treatment of diabetes-associated neuropathy.
ROLE OF THE P2X7 RECEPTOR IN THE ALTERED CALCIUM HOMEOSTASIS OF SCHWANN CELLS OVEREXPRESSING PERIPHERAL MYELIN PROTEIN 22 (PMP22)


Objective of our study is to evaluate the involvement of the P2X7 purinergic receptor in the regulation of free calcium concentration ([Ca2+]i) in Schwann cells (SC) isolated from the rat model of Charcot-Marie-Tooth 1A (CMT1A) disease. In fact, we found abnormally high intracellular [Ca2+]i in CMT1A SC. Interestingly, interaction of PMP22 with the C-terminal domain of the P2X7 purinergic receptor leads to receptor activation, channel opening and Ca2+ influx. First, we studied PX27 expression in CMT1A and control SC by semiquantitative RT-PCR, and stimulated SC with millimolar ATP, a condition known to trigger P2X7-mediated Ca2+ influx. Then, to assess the replenishment state of the intracellular calcium stores in the cells, we tested the effect of thapsigargin (TG), which is known to empty the cADPR- and IP3-sensitive endoplasmic reticulum (ER) Ca2+ stores by inhibiting ATP-dependent Ca2+ pumps. Finally, to evaluate the direct role of P2X7 in modulating ([Ca2+]i) in CMT1A SC, we pre-incubated the cells with 20 microM KN-62 and oATP, which are selective inhibitors of this receptor. CMT1A SC expressed significantly higher levels of P2X7 mRNA than normal controls. In line with this observation, stimulation of SC with millimolar ATP consistently induced a higher increase of [Ca2+]i in cells overexpressing PMP22 compared to controls. Moreover, the Ca2+ content of the TG-sensitive stores was significantly reduced in SC from CMT1A rats compared to controls. Finally, treatment of CMT1A SC, but not of normal ones, with P2X7 inhibitors reduced the [Ca2+]i to levels comparable to those measured in normal SC. As Increased calcium levels have been already demonstrated to be responsible for impairment of SC differentiation and myelination, but the mechanisms underlying the [Ca2+]i increase are so far undetermined, identification of mechanism and related pharmacological tools for restoring normal SC calcium levels could lead to the development of new therapeutic approaches to CMT1A and possibly to other neuropathies sharing a common derangement of SC calcium homeostasis.
ACETYL-L-CARNITINE (ALC) MAY PREVENT AXONAL IMPAIRMENT IN A RAT MODEL OF CHARCOT-MARIE-TOOTH (CMT) TYPE 1A DISEASE


*Dip. Neuroscienze, Oftalmologia e Genetica - Università di Genova, ** Centro Neurofisiologia del Dolore - Università di Genova, *** Dip. Scientifico, Sigma Tau - Pomezia

CMT1A is a common hereditary neuropathy characterized by demyelination and a severe axonal impairment. ALC is a member of the carnitines family, able to stimulate energy metabolism and to exert a putative neuroprotective role in several experimental paradigms. We studied the effect of ALC on the electrophysiological performances of a CMT1A rat model. We treated three littermates of five week-old CMT1A and wild type rats daily, with a subcutaneous injection of ALC 100mg/kg or vehicle for 35 days. All the animals have been evaluated for motor and sensory nerve conduction velocities (MNCV and SNCV) and amplitude of evoked potentials of the tail nerve. In fact, these nerve, never studied in CMT1A rats so far, provides an excellent opportunity for access to the longest peripheral nerves and is very easily and precisely measurable. At baseline, a significant difference in all the neurophysiologic measurements was observed between CMT1A and control rats, suggesting that recording from the tail nerve gives similar results to the sciatic nerve. Both treated and untreated normal rats showed the expected increase in MNCV and SNCV, and amplitude of evoked potentials due to developmental nerve growth, thus suggesting that ALC is not detrimental at this time of administration. In treated CMT1A rats we observed an increase of the motor action potential amplitude of 59.4% vs an increase of 4.9% recorded in the animals treated with the vehicle. Similarly, we observed an increase of the 95% of the motor action potential area in the pathological rats after ALC treatment vs an increase of 65% in the control animals. On the contrary, we did not observe any effect on motor conduction velocities nor on all the different sensory parameters in treated vs untreated CMT1A rats. In conclusion, these preliminary results indicate that ALC may act as a protective agent for CMT1A associated motor axonal sufferance, at least stimulating axonal growth to a rate comparable to that of control animals.
ROLE OF THE EXTRACELLULAR MATRIX IN REGENERATING AND NON-REGENERATING AXONAL NEUROPATHIES


*Neuropathology Unit and Dept. Neurology, ** Dept. Neurology, ***Human Hereditary Neuropathy Unit, San Raffaele Scientific Institute - Milan

Our knowledge of the regulatory mechanisms and signaling cascade underlying the axonal regeneration program in human peripheral nervous system is still very limited. Furthermore the majority of the molecular players involved in nerve regeneration await identification. The extracellular matrix (ECM) in the endoneurium plays a crucial role in nerve regeneration.

We selected nerve biopsies from patients with peripheral axonal neuropathy that were assigned in two distinct groups by the presence of efficient axonal regeneration or the absence of axonal regeneration, independently from the etiology. We investigated differences in the expression of several ECM components, including laminins, collagen, fibrin(ogen), fibronectin and vitronectin.

Our results showed different ECM composition in the two groups. Patients with efficient axonal regeneration presented high expression of fibronectin while vitronectin and fibrin(ogen) were almost absent. Instead, patients with absence of axonal regeneration showed low expression of fibronectin and high expression of vitronectin and fibrin(ogen).

Fibrin and vitronectin would constitute a primordial ECM necessary for immediate repair after damage. The subsequent transformation of the primordial ECM into mature ECM mainly composed by fibronectin is necessary for adequate and efficient nerve regeneration. Persistence of fibrin and vitronectin reflects defective/inappropriate ECM degradation that may cause defective nerve regeneration. Our result would suggest that different ECM composition might influence the outcome of axonal peripheral neuropathies.
RESULTS OF GLUTAMATE CARBOXYPEPTIDASE II (GCP II, NAALADASE) INHIBITION IN EXPERIMENTAL CISPLATIN-INDUCED PERIPHERAL NEUROTOXICITY


Glutamate Carboxypeptidase II (GCP II) is a metallopeptidase in the central and peripheral nervous systems responsible for cleaving the abundant dipeptide N-acetyl-aspartyl glutamate and liberating glutamate. Central and peripheral nervous system injuries are less severe in mice lacking the Folh1 gene encoding for GCP II. It has been demonstrated that the pharmacological inhibition of GCP II can both prevent and treat the peripheral nerve changes observed in both BB/W and chemically induced diabetic rats.

We have designed an experimental program with the following aims: 1) to investigate whether GCP II inhibition interferes with the anticancer effect of platinum-derived drugs and taxanes, 2) to explore the putative neuroprotective effect of GCP II inhibition in a well-established cisplatin-model of peripheral neurotoxicity and 3) to determine the distribution of glutamate transporters (GT) GLT-1, GLAST and EAAC1 and of GCP II in the DRG and peripheral nerve.

In vivo studies performed using human colon C51 and human ovarian OVCAR-3 flank xenograft models demonstrated that GCP II inhibitors do not interfere with the antitumor effect of different anticancer drugs, even at very high doses. In the cisplatin model GCP II inhibition induced a significant protection against the tail nerve conduction velocity reduction (ctrls = 33.9+/−2.3 m/sec, cisplatin = 22.6+/−3.6 m/sec, cisplatin + GCP II inhibitor GPI16072 = 27.0+/−4.7 m/sec, p<0.01 vs. cisplatin alone). This protective effect was confirmed also by the morphometric examination performed on somatic, nuclear and nucleolar size of DRG neurons. The study on the distribution of GT and of GCP II evidenced that under physiologic conditions all these molecules were detectable by immunoblotting in the DRG and sciatic nerve. The subsequent preliminary immunolocalization study suggests that most of these molecules can be observed in the DRG satellite cells and in Schwann cells.
PLATINUM-TAXANE COMBINATION CHEMOTHERAPY: GENERAL AND PERIPHERAL NEUROTOXICITY OF THEIR COMBINED ADMINISTRATION IN THE RAT


Animal models represent a reliable and useful tool to investigate the mechanisms of chemotherapy-induced peripheral neurotoxicity (CIPN) and they are widely used to explore the potential role of neuroprotectants and the effect of different administration schedules on the course and severity of CIPN. However, despite single-agent chemotherapy is definitely rare in human cancer treatment, all the animal models reported so far are based on single-agent schedules.

We report the first study performed using in the Wistar rat the combined administration of the first-in-class of taxane and platinum drugs with a schedule based on the sequential administration of different doses of paclitaxel (P, iv once weekly) and cisplatin (CDDP, ip once weekly). The general toxicity of the combined treatment and its neurotoxicity were assessed after 4 weeks and the results were compared with the results of healthy control rats and those of rats treated with cisplatin or paclitaxel alone.

No mortality was observed in the dose range used in our study for both compounds (i.e. 5-10 mg/kg for P and 3-4 mg/kg for CDDP). P+CDDP administration induced a small reduction in weight gain vs. control rats and vs. single agent-treated rats. Hematological and blood chemistry changes were not significantly different in the co-treated animals vs. controls rats. At sacrifice no macroscopic changes involved the thoracic, abdominal or pelvic organs. Mild tubular damage in the kidney and bone marrow hypoplasia were the most evident pathological changes at the light microscope. Regarding neurotoxicity, a significant and dose-dependent reduction in nerve conduction velocity was observed after P+CDDP administration. These changes were paralleled at the pathological examination by reduction in dorsal root ganglia neuron soma, nuclear and nucleolar size, by axonal damage in the sciatic nerve and by reduction in intraepidermal nerve fiber density.

This study established for the first time a P+CDDP model of CIPN which mimics in a realistic way the human use of these effective antineoplastic agents.

Supported in part by a “Fondazione Banca del Monte” unrestricted research grant.
Epothilone is a new antineoplastic compound extremely active as a microtubule stabilizer. Since its discovery, it has been recognized that the effect of epothilone is superior to the effect of paclitaxel or docetaxel and, moreover, epothilone is effective against taxane-resistant tumor cells. At the moment the major limitation in the use of epothilone and epothilone analogues is a dose-limiting peripheral neurotoxicity.

We report the first ever reported in vitro and in vivo examination of the neurotoxicity of EPO-B. The in vitro study, performed using E15 rat DRG explants, evidenced that in the range 0.1 nM-1 μM at EPO-B concentration higher than 50 nM a significant (i.e. more than 50%) reduction was observed after 24 and 48 hours. In the in vivo study, tail nerve conduction velocity (NCV) was serially assessed in a range of doses between 0.25 and 2 mg/kg IV 1q7d x 4w. The animals treated with EPO-B 2 mg/kg did not complete the treatment period because of dose-limiting general toxicity, but also in the EPO-B 1.5 mg/kg mortality was observed. EPO-B at the dose of 0.25, 0.5 and 1 mg/kg induced a significant decrease of the NCV at the end of the treatment (mean values 20.8 m/sec, 21.8 m/sec and 20.6 m/sec respectively, vs. 27.8 m/sec in vehicle-treated and 30.7 m/sec in untreated controls, p < 0.001 for all EPO-B schedules vs. both control groups).

At the pathological level the preliminary morphological observation performed on the sciatic nerve of EPO-B-treated rats evidenced axonal damage as the main pathological change.

These experimental results will be helpful for further studies on the mechanism of action of EPO-B and for comparison with newly available EPO-B analogues.
LYMPHATIC VESSELS IN HUMAN SURAL NERVE. AN IMMUNOHISTOLOGICAL AND MORPHOLOGICAL STUDY


Investigations on lymphatics have been greatly enhanced by identification of specific markers of lymphatic endothelium such as LYVE-1, podoplanin, prox-1 (1) and D2-40 (2), whereas characterization previously relied only on ultrastructural criteria. Lymphatic vessels are localized in most districts with the exception of central nervous system, bone marrow, cartilage and cornea. Very few studies deal with presence of lymphatic vessels in peripheral nerves, that would be expected in epineural connective environment surrounding nerve bundles (3). Experimental data (4) indicate that lymphatics are involved in removal of post-phagocytic endoneurial macrophages from injured nerve in Wallerian degeneration. We investigated the presence of lymphatics by the different available markers in human sural nerve. Bioptic sural nerve specimens were obtained in cases of inflammatory and non-inflammatory neuropathies and from normal subjects. Ultrastructural examination identified lymphatics by: irregular endothelial profile, discontinuous or absent basal membrane, anchoring filaments, absence of pericytes and numerous pinocytotic vesicles. Small lymphatics were also observed at the periphery of perineurium. For immunohistological identification of lymphatics, immunoperoxidase reaction was carried out with D2-40, LYVE-1 and podoplanin, in paraffin embedded or snap frozen sections. Lymphatics were detected adjacent to blood vessels or scattered in epineurium but not in endoneurium. Perineurium and Schwann cells also resulted D2-40 and podoplanin reactive, whereas no localization was observed in these cells by LYVE-1. Blood vessels endothelium was not immunoreactive for any marker whereas smooth muscle cells and elastic fibers were LYVE-1 positive. According to the demonstration in experimental models that lymphangiogenesis is triggered by macrophages, via VEGF-C/D production (5), immunohistochemical detection in human peripheral nerve will be a tool for definition of the contribute of lymphatics in nerve pathophysiology.

2. Kahn JH, Lab Invest 82 (2002), 1255-1257
BORTEZOMIB-INDUCED NEUROPATHY: RESULTS OF A DOSE-CORRELATION STUDY


*Dept. Neurosciences and Biomedical Technologies, ** Dept. of Hematology, Osp. S. Gerardo - University of Milano Bicocca - Monza

Bortezomib, the first proteasome inhibitor available in clinical practice, is a very promising new generation anticancer drug. Although, in most cases, sensory neuropathy is a manageable side effect in some patients it can be severe and require a reduction of the dose or even treatment withdrawal. The current knowledge regarding this side effect is still unsatisfactory and the long-term course of the neuropathy symptoms and signs is unclear.

We serially evaluated 28 second-line bortezomib-treated myeloma patients with the clinical Total Neuropathy Score (cTNS) and with a battery of neurophysiological tests (sensory and motor ulnar nerve, peroneal and sural nerves). So far, 23 patients have been evaluated at least once after the beginning of the treatment (bortezomib dose range 7.9 to 43.1 mg/m²) and 6 were examined 5 to 9 months after treatment withdrawal.

The cTNS score at baseline range was 0-11 (median = 4) and all but 1 patient had at least a score = 1. As expected the overall final cTNS increased (range 1-14, median 7), mostly due to the occurrence of sensory symptoms and signs, but a significant dose-relationship was not demonstrated using the Spearman r test. When the absolute neurophysiological values were evaluated, in 6 patients (3 with normal and 3 with abnormal baseline values) the sural nerve was no longer elicitable after different doses of bortezomib. With this possible bias, no correlation was found with any neurophysiological parameter and the dose of bortezomib administered. Pain was a major complaint requiring symptomatic treatment in 5 patients. At the follow-up examination 2 patients were stable, 2 had a reduction in their cTNS (4 and 6 point respectively) and 2 had a 1 point cTNS worsening. Two of these patients had painful symptoms which were no longer present at the follow-up examination.

These data indicate that 1) the severity of bortezomib neuropathy can markedly differ among patients with pre-existing neuropathy, 2) pain is frequent during treatment and 3) recovery is uncertain, thus supporting the usefulness of further investigation of first-line patients.