

Rodent Models of Chemotherapy-Induced Peripheral Neuropathy

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Abstract

Peripheral neuropathy is a common and dose-limiting side effect of many chemotherapeutic drugs. These include platinum compounds, taxanes, vinca alkaloids, proteasome inhibitors, and others such as thalidomide and suramin. Although many rodent models have been developed using either mice or rats, there is limited consistency in the dose or mode of delivery of the drug; the sex, age, and genetic background of the animal used in the study; and the outcome measures used in evaluation of the peripheral neuropathy. Behavioral assays are commonly used to evaluate evoked sensory responses but are unlikely to be a good representation of the spontaneous sensory paresthesias that the patients experience. Electrophysiologic tests evaluate the integrity of large myelinated populations and are useful in drugs that cause either demyelination or degeneration of large myelinated axons but are insensitive to degeneration of unmyelinated axons in early stages of neuropathy. Histopathologic tools offer an unbiased way to evaluate the degree of axonal degeneration or changes in neuronal cell body but are often time consuming and require processing of the tissue after the study is completed. Nevertheless, use of drug doses and mode of delivery that are relevant to the clinical protocols and use of outcome measures that are both sensitive and objective in evaluation of the length-dependent distal axonal degeneration seen in most chemotherapy-induced peripheral neuropathies may improve the translational utility of these rodent models.

Key Words: axon degeneration; bortezomib; chemotherapy; cisplatin; intraepidermal nerve fiber density; paclitaxel; peripheral neuropathy; vincristine

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Introduction

A common complication of chemotherapy is neurotoxicity that often manifests itself as peripheral neuropathy. Many cancer drugs can cause chemotherapy-induced peripheral neuropathy (CIPN), and the incidence can be up to 90%. Although CIPN is a dose-limiting side effect of chemotherapy, its clinical course is similar to other toxic neuropathies. A significant percentage of patients improve once the offending drug is stopped, but as much as 50% of the affected individuals are left with residual peripheral neuropathy that affects their quality of life. Clinical features, diagnosis, and management of CIPN have recently been reviewed and will not be repeated here (Grisold et al. 2012). Instead, this review will focus on rodent models of CIPN, with special attention to outcome measures used to evaluate the effects of chemotherapy on the peripheral nervous system, and will critically review how faithful these models are to the human disease.

Outcome Measures Used in Evaluation of Neuropathy in Rodent Models of CIPN

Several behavioral, electrophysiologic, and pathologic methods are used as outcome measures to evaluate neuropathy in rodent models of CIPN. Most rodent models published so far have used only a subset from this wide selection of outcome measures. Most of these outcome measures are chosen to evaluate symptoms or signs seen in CIPN patients, but as outlined below, many of them have significant shortcomings in terms of relevance to the actual symptoms and examination findings in patients.

Behavioral Testing

There are several behavioral tests used to assess various symptoms associated with neuropathy. These tests have been developed to assess forms of sensory perception, including various forms of evoked pain and sensory-motor coordination.

Mechanical Allodynia

Mechanical allodynia refers to an exaggerated painful response to mechanical stimulation. It is most commonly

assessed using monofilaments called the von Frey monofilaments. This test involves touching the hind midplantar paw with a series of eight monofilaments, increasing in stiffness (Chaplan et al. 1994). The von Frey filaments are applied perpendicular to the surface with force that will cause the filament to arch for roughly 6 to 8 seconds, which, in healthy animals, results in the paw being sharply withdrawn. Paw withdrawal threshold can be measured to assess mechanical allodynia. Results are measured in two ways: either a specific von Frey filament is selected and the percentage of animals that withdraw is recorded or the series of filaments are used and the one causing paw withdrawal is recorded.

Mechanical Hyperalgesia

The paw pressure test involves increasing pressure on the inflamed paw of a mouse or rat (Randall and Selitto 1957). Inflammation is typically induced by applying dry yeast under the skin of the plantar surface of the hindlimb. The test is usually conducted using an Analgesy meter that has been fitted with a weight between 0 and 1000 g and applied at a rate of 64 g/s. A number of thresholds can be used, including the animal's vocalization and vigorous paw removal attempt. Some studies will use a modified method in which pressure is applied to a healthy paw rather than an inflamed one (Leighton et al. 1988).

Thermal Hypo- or Hyperalgesia

Thermal sensation can be determined using a number of methods, including tail immersion, radiant heat assay, tail-flick test, or cold plate assay. The tail immersion test involves immersing the tail of the mouse or rat into cold or hot water at either 4°C and 46°C (noxious temperatures) or 10°C and 42°C (nonnoxious temperatures) until the animal withdraws its tail (Necker and Hellon 1978). The duration of the tail immersion is recorded for comparison.

The radiant heat assay is performed by placing the animals on a glass surface with a consistent temperature of 30°C or 25°C and a thermal stimulus in the form of a light source that can be manually used to deliver heat to a hindpaw of the animal (Hargreaves et al. 1988). A timer that is linked to the light source is used to measure the response latency or time necessary for the animal to abruptly withdraw its paw.

The tail-flick test is completed by using a bulb with a reflector to focus light on the tip of a rodent tail, which has been positioned in a grooved board. The set up allows for the light and stop watch to be turned on simultaneously and turned off with the flick of the rodent's tail, which occurs when the animal experiences pain induced by heat (D'Amour and Smith 1941).

The cold plate assay is performed using a cold plate usually set to $-4.2^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The rodent is placed on the plate, and hypersensitivity is typically measured by counting the number of times the animal jumps or either hindpaw is lifted rapidly over an allotted period of time (usually not exceeding

5 minutes). Accuracy is usually ensured using a camcorder to record the test, with the video replayed in slow motion (Ta et al. 2010).

Sensory–Motor Coordination

Overall sensory–motor coordination can be evaluated using a device (Rotarod) that requires both proprioceptive sensory function and motor strength. Rotarod testing involves placing a rodent on a rotating rod, which is suspended high enough to prevent the animal from wanting to fall, yet low enough that falling does not cause injury. Speed can be kept constant or accelerated (Jones and Roberts 1968). Studies will typically select a constant speed and time lapse to evaluate how long the animals were able to remain on the rod.

Electrophysiological Testing

Although behavioral testing has become common in studies using rodent models of CIPN, it is not exclusive and it is not always the sole choice of evaluation. There are also a number of electrophysiologic methods that can be used to evaluate CIPN. The methods used for conducting electrophysiologic testing in rodent models are based on those used in the clinic; they have been scaled down to fit rodent models (Sullivan et al. 2008). Primarily, nerve conduction studies are used to measure the electrical conduction of both motor and sensory nerves. Nerve conduction studies provide a measurement of amplitude and latency of the evoked response, which can then be used to calculate nerve conduction velocity.

Motor Nerve Conduction Studies

A compound muscle action potential can be used to evaluate peripheral nerve conduction based on evoked motor response and can therefore be used as a measure of motor neuropathy. It is performed by stimulating a peripheral nerve, typically the sciatic nerve, by placing stimulating electrodes at the sciatic notch and recording electrodes at the distal sciatic nerve–innervated muscles in the foot or leg below the knee. Amplitude of the response is calculated and gives a measure of the number of large myelinated motor axons in the nerve. A second stimulation at the knee provides a distal stimulation site and allows one to calculate motor nerve conduction velocity (Xia et al. 2010). An important consideration is that nerve conduction studies are susceptible to the effects of various anesthetics used in such studies, and these should be appropriately recorded and reported (Oh et al. 2010).

Sensory Nerve Conduction Studies

Sensory nerve action potential is measured in the same way as compound muscle action potential except a solely sensory

nerve is used; thus it is a measure of purely sensory nerve conduction. This is often done in the tail sensory nerve by stimulating at the base of the tail and recording at a fixed distal site along the tail. Sensory nerve action potential amplitudes are on a much smaller scale than those seen in a compound muscle action potential and often require averaging of multiple stimulations to obtain a reliable evoked response.

Although these techniques are commonly used to confirm nerve degeneration in comparison with vehicle control animals, results vary greatly among studies done in different laboratories and even in a given laboratory. There are many reasons for such high variability, but a common problem is that many laboratories use changes in nerve conduction velocities as one of the most reproducible outcome measures yet do not often control for temperature during the recordings. This often affects the baseline at which comparisons are made (Rebert et al. 1984). However, the most significant shortcoming of the conduction velocity changes is that nerve conduction velocities do not correlate with symptoms. In patients with neuropathies where there are reduction in conduction velocities, symptoms often correlate with secondary axonal loss rather than small changes in conduction velocities of motor or sensory fibers (Krajewski et al. 1999). Given this observation in humans, in most animal studies, where investigators have shown small, statistically significant changes in nerve conduction velocities, the biological significance of these observations is unclear.

It is also common to see variations in how frequently the electrophysiologic parameters are evaluated after induction of neuropathy by a chemotherapy agent. A consensus is required to simplify and standardize electrophysiologic studies in rodent models of CIPN. Not all studies will consist of both behavioral and electrophysiologic testing, but they usually conduct a series of one or the other.

Histologic Analyses

Histopathologic changes in rodent models of CIPN often focus on degeneration of neurons and/or axons. Changes in neuronal morphology and axonal degeneration are evaluated using tissue sections either stained with immunohistochemical methods (against neuronal markers) or sections embedded in plastic and stained with Toluidine blue for better ultrastructural analysis. It is important to note that histologic evaluations need be completed using stereologic methods. Often most investigators count axons or neurons by using profile counts, with or without blinded random sampling method, rather than using true serial reconstructions or stereologic methods (Coggeshall and Lekan 1996).

Evaluation of Neuronal Morphology and Numbers

Counting neurons provides a method for evaluating neuronal loss associated with the severity of neuropathy brought about by disease or experiment (Coggeshall and Lekan 1996). The effect of chemotherapy on neuronal cell bodies is often eval-

uated by analyzing random cross-sections of dorsal root ganglia typically chosen from three different lumbar ganglia (i.e., L4–L6) (Cavaletti et al. 1992). Several different characteristics of neurons are evaluated. These include counting neurons with nuclei, neurons with nucleoli, neurons with multiple nucleoli, or neurons with nuclear eccentricity (Tomiwa et al. 1986).

Nerve Morphometry

Because the general underlying pathology of CIPN is axonal degeneration, evaluation of axons in a peripheral nerve can be used to determine the degree of neuropathy. A number of morphometric observations can be made by selecting fields at random; axon numbers can be counted, and axonal atrophy and state of myelination can be determined (Cavaletti et al. 1992). Degenerating axons are often distinguished by presence of myelin ovoids and lack of axoplasm (Wang et al. 2002). Axon density can be determined by dividing the number of intact axons by the area of the tissue section. Measurement of the axon and fiber diameters of the myelinated fibers is used to calculate the g ratio, which is a useful index of myelination. Axon counting is most reliable when it is done using stereologic methods (Coggeshall and Lekan 1996). These different forms of axon quantification are the most common and effective practices for measuring neuropathy.

Intraepidermal Nerve Fiber Density

Integrity of the most distal parts of unmyelinated sensory axons can be determined through evaluation of skin punch biopsies. In rodent models of CIPN, plantar foot pads are harvested by punch biopsy, then sectioned at 50- μ m thick sections on a freezing microtome, and stained with a pan-axonal marker, PGP 9.5 antibody. Intraepidermal unmyelinated axons are counted blindly, and the intraepidermal nerve fibers density per millimeter of skin is determined (McCarthy et al. 1995). This method of evaluation is a repeatable, and thus reliable, technique used to quantify unmyelinated fibers (Holland et al. 1997).

Commonly Used Animal Models of CIPN

Most of the chemotherapeutic drugs that cause peripheral neuropathy in cancer patients have been used to develop rodent models of CIPN. Commonly used ones include platinum compounds, taxanes, proteasome inhibitors, vinca alkaloids, and others, including thalidomide and suramin (Table 1). However, there are presently a number of issues with modeling CIPN in animals. First, there is little consistency in the type of animal used across laboratories; with rodents, both mice and rats of different sex, age, mass, and genetic background have all been used. It is possible that these variables play a role in the inconsistencies of behavioral testing results between laboratories (Hoke 2012). Another

Table 1 Chemotherapy agents commonly used in animal models of chemotherapy-induced peripheral neuropathy

Platinum compounds	Taxanes	Vinca alkaloids	Proteasome inhibitors	Others
Cisplatin	Paclitaxel	Vincristine	Bortezomib	Thalidomide
Carboplatin	Docetaxel		Carfilzomib	Suramin
Oxaliplatin				

major issue with these models is the matter of dosing discrepancies: mode of administration, dose, and duration. These, like evaluation, vary greatly among studies to the extent that laboratories attempting identical dosing report varying results. In these cases, the most likely explanation for these inconsistencies is the genetic background of the animals used for the study (see Taxanes, below).

Platinum Compounds

Platinum compounds have been used in chemotherapy regimens for more than 40 years and continue to cause CIPN and limit dosing (Windebank and Grisold 2008). Rodent studies investigating CIPN induced by platinum compounds vary greatly in their methods. As mentioned before, there is limited consistency in whether mice or rats are used, their age, weight, or genetic background. Additionally, there are great variations in how the animals are dosed with compounds and for how long. Despite studies completed to determine effects of different dosing schedules on the peripheral nerves and the majority of publications stating that dosage and duration of drug administration are based on what is clinically relevant, major discrepancies remain.

One thing that the majority of studies involving platinum compound-induced peripheral neuropathy have in common is the mode of drug administration, which is usually intraperitoneal injection. Unfortunately, intraperitoneal injections are not the best method to model the human disease because it is not a mode of delivery in patients because such injections result in a local toxic effect. The ideal mode of administration would be intravenously by tail vein injection.

In the case of cisplatin, it is consistently diluted in saline and administered via intraperitoneal injection. Studies have been done in both mice and rats with varying dosing schedules. There is a history of discrepancies in results from different laboratories conducting very similar analyses. For example, in one instance a laboratory dosed mice with 10 mg/kg cisplatin once each week for 2 months, but another laboratory, using an identical dose and duration, could not complete dosing the animals for 8 weeks because of high mortality rate (Apfel et al. 1992; Verdu et al. 1999). This disparity may have been due to one study using CD1 male mice and the other using Swiss female mice. Differences in genetic, sex, and/or age variations were not investigated; instead, a lower dose of cisplatin was used for treatment. More recently,

dosing schedules have shifted from once per week for several weeks to cycles of 1 week of intraperitoneal injections followed by 1 week of rest.

Taxanes

Docetaxel and paclitaxel (generically known as taxol) are examples of taxanes, a group of chemotherapeutic drugs extracted from the bark of *Taxus brevifolia*. Taxol interferes with the interaction of microtubule-associated proteins (Black 1987). Although this antitumor activity makes it a good chemotherapy agent, it is also toxic to neurons and cause peripheral neuropathy (Rowinsky et al. 1993).

Mode of Administration, Dose, and Duration

The earliest models of paclitaxel-induced neuropathy were in rats. Several early investigations of paclitaxel-induced neuropathy used local subperineurial injection, which resulted in demyelination, axonal swelling, and distal degeneration (Roytta and Raine 1985, 1986). It was obvious that these models were not an accurate representation of the human disease. Other modes of administration for this form of CIPN include intraperitoneal or intravenous injections. Repeat intraperitoneal injections are often used, simulating chemotherapy treatment (Cavaletti et al. 1995). High-dose treatments of 16 mg/kg of paclitaxel over 5 weeks in Wistar rats resulted in moderate axonal degeneration, swelling of mitochondria, and minimal demyelination. This dosing structure, compared with intravenous dosing, is very mild. Intravenous administration, at even two low doses of 12 to 18 mg/kg 3 days apart in Sprague-Dawley rats, induces severe large fiber sensory neuropathy. Axonal degeneration and hypomyelination in dorsal roots are seen, as well as slowed motor and sensory nerve conduction velocity (Cliffer et al. 1998). The large fiber deformities in these intravenously treated animals were present for up to 4 months. Although intravenous dosing creates a neuropathy that is more clinically relevant, the majority of studies continue to use intraperitoneal injection as the mode of administration.

The mode of administration for paclitaxel-induced neuropathy in mice varies, with some investigators using intraperitoneal injection and others injecting intravenously. As mentioned before, intravenous administration is ideal; it seems as though more laboratories are using intravenous

injections for taxol-induced neuropathy studies than for studies with platinum compounds.

In the case of paclitaxel, it is consistently prepared with Cremophor EL and ethanol and diluted with saline. There are, however, inconsistencies with the doses and the dose schedules, in addition to administration method. For instance, one recent study used paclitaxel through intraperitoneal injection once daily for 5 consecutive days, reaching a cumulative dose of 10 mg/kg in CD1 male mice (Ruiz-Medina et al. 2013). Other studies have treated female C57 mice with three 30 mg/kg intravenous injections every other day (Wang et al. 2002; Wang et al. 2004). One laboratory used a low dose of paclitaxel (3–30 mg/kg intravenous injections) in one group of mice and a high dose (3–60 mg/kg intravenous injections) in another and reported the low-dose animals as having little axon degeneration with inconsistent results (Wang et al. 2004). This is obviously very different from the group that reported peripheral neuropathy in mice with a cumulative dose of only 10 mg/kg (Ruiz-Medina et al. 2013). This discrepancy may be because of the difference in age of animals, which was not reported in one of the studies, or the differences in sex, genetic background, or mode of administration. Another study used 8-week-old female AJ mice and a lower dose of paclitaxel (25 mg/kg) administered intravenously by tail vein injection every other day for 3 doses and demonstrated reduction in intraepidermal nerve fiber density as the primary outcome measure (Melli et al. 2006).

Age and Genetic Background

Taxol studies have shown great variation in degree of peripheral neuropathy based on the genetic background of mice, as well as the age of animals used. Despite these results, there is still little consistency in the genetic background of mice used for taxol-induced neuropathy studies.

A pivotal study demonstrated the impact of sex and genetic background on development of neuropathy symptoms in a model of taxol-induced neuropathy (Smith et al. 2004). In this study, investigators administered a cumulative dose of 4 mg/kg of paclitaxel through 4 intraperitoneal injections every other day in 10 different inbred mouse strains while measuring mechanical and cold allodynia and thermal hyperalgesia. The results showed statistically significant variations in mechanical allodynia among all strains, with some exhibiting very robust allodynia and others minimal. The study established DBA/2 as a high-allodynic strain and C57BL/6 as a low-allodynic strain. However, despite their significant variations in mechanical allodynia, these strains had comparable cold allodynia and did not display any thermal hyperalgesia. It was additionally noted that male mice had more prominent allodynia than female mice and that the opposite has been observed in Sprague-Dawley rats in the past (DeLeo and Rutkowski 2000; Smith et al. 2004). One shortcoming of this study was the lack of correlation of behavior with histologic measures of peripheral neuropathy.

Another study that involved administration of paclitaxel (10 mg/kg cumulative dose) to 31-day-old (young), 3 to

4-month-old (adult), and 12 to 13-month-old (aged) CD1 mice evaluated the impact of age in taxol-induced neuropathy (Ruiz-Medina et al. 2013). The results showed that young mice, followed by aged mice, had more severe impairment of mechanical allodynia and thermal hyperalgesia. Thus, the age of the animals used affects the level of neuropathy and needs to be standardized. Unfortunately, it is common for publications to not even include the exact age of the animals used for the experiments.

Vinca Alkaloids

Vinca alkaloids, derived from a common ground plant (the periwinkle plant, *Catharanthus roseus*), act as chemotherapy agents by inhibiting microtubule assembly and promoting its disassembly (Himes et al. 1976). Unfortunately, vincristine, a vinca alkaloid commonly used to treat cancers, is severely neurotoxic; painful neuropathy that develops with vincristine-containing chemotherapy is a dose-limiting side effect (Armstrong et al. 1967).

The first animal models of vincristine-induced peripheral neuropathy were completed using rats (Aley et al. 1996; Boyle et al. 1996). Attempts to create a mouse model were made later, but mice are not used as commonly as rats (Nakamura et al. 2001; Uceyler et al. 2006).

Initial rat models of vincristine-induced neuropathy followed similar doses and schedules of daily injections, Monday through Friday, for 2 weeks, which was similar to what was done in the clinic (Aley et al. 1996; Boyle et al. 1996). These early studies established a dose of 0.1 mg/kg injections sufficient for inducing clinically relevant symptoms in rats. There are, however, variations in how vincristine is administered, with some laboratories using intraperitoneal injections and others using intravenous administration. There are also variations in how vincristine is given intravenously. Most common mode is an intravenous injection through the tail vein; however there have been studies completed using a mini-osmotic pump to supply vincristine continuously over 2 weeks, providing the rats with a uniform blood concentration (Nozaki-Taguchi et al. 2001). The long-term neuropathic pain associated with continuous infusion is very similar to the pain experienced by human patients (Higuera and Luo 2004). In most recent studies, doses vary between 0.05 mg/kg and 0.15 mg/kg, with some laboratories administering either intravenously or by intraperitoneal injection over the course of 2 weeks (Jaggi and Singh 2012; Sweitzer et al. 2006; Wala et al. 2012). The majority of these studies used adult, male Sprague-Dawley rats, but there are some studies that used female DA rats or Wistar rats (Aley et al. 1996; Authier et al. 2003; Boyle et al. 1996; Nozaki-Taguchi et al. 2001; Thiagarajan et al. 2013).

When mice are used as a model for vincristine-induced neuropathy, there is even less consistency in dosing; the doses used range from 0.1 mg/kg to 2 mg/kg (Apfel et al. 1993; Saika et al. 2009). There is also great variation in the schedules used for administering vincristine in mouse models. In

some cases, the same clinically relevant schedule used in rat models (treat for 5 days, skip 2 days, treat for another 5 days) is used (Uccyler et al. 2006). Other studies deviate from this schedule, dosing twice each week for a period of 8 to 10 weeks or daily for 7 days (Apfel et al. 1993; Contreras et al. 1997; Saika et al. 2009). These studies also vary in the genetic background of the mice used for testing.

Proteasome Inhibitors

Proteasome inhibitors were found to result in both antiproliferative and proapoptotic activity against tumors in vitro (Drexler 1997; Imajoh-Ohmi et al. 1995; Shinohara et al. 1996). Bortezomib was the first proteasome inhibitor to enter the clinic, swiftly advancing through trials and obtaining approval for treatment of multiple myeloma (Jagannath et al. 2004; Kane et al. 2006; Orlowski et al. 2002). Bortezomib acts by inhibiting the 26S proteasome, which is part of the ubiquitin degradation pathway, ultimately downregulating expression of proteins that usually promote cell proliferation. Other proteasome inhibitors are presently in clinical trials. Carfilzomib recently went through a similar rapid approval process and does not seem to cause the severe neuropathies seen with bortezomib (Zhang et al. 2013). Meanwhile, the peripheral neuropathy that develops with bortezomib treatment is the dose-limiting side effect.

Because proteasome inhibitors are a fairly novel form of chemotherapy, the associated neuropathy is not entirely understood. The first attempts at modeling this type of CIPN with bortezomib were done in rats, followed by very recent studies that tried to characterize a mouse model (Bruna et al. 2010; Cavaletti et al. 2007; Meregalli et al. 2010).

Some issues with the original studies that tried to create an accurate rat model involved the lack of neurophysiologic results in human patients, which prevented a comparison with a relevant, established model (Cavaletti et al. 2007). These initial studies established a dose of 0.3 mg/kg of bortezomib twice each week as an intolerable dose, resulting in death in female Wistar rats. A similar investigation using the same type of animals found a dose of 0.2 mg/kg 3 times each week for 4 weeks was tolerable, with only one death reported (Carozzi et al. 2010).

Similar to the case with platinum compounds and taxanes, as models of bortezomib-induced neuropathy are developed, they too lack consistencies in mode of administration and dosing schedule. As already stated, the ideal mode of administration for these studies is intravenous injection, mimicking the mode of delivery in patients. Although there is a trend of using adult, female Wistar rats, two recent studies varied in almost every aspect of their models (Cavaletti et al. 2007; Meregalli et al. 2010; Meregalli et al. 2012; Zheng et al. 2012). In these studies, one group administered 0.2 mg/kg intraperitoneal injections to adult, female Sprague-Dawley rats once a day for 5 consecutive days for a cumulative dose of 1 mg/kg with no deaths (Zheng et al. 2012). The other

study involved the same dose (0.2 mg/kg) of bortezomib administration to female, adult Wistar rats but with intravenous injections 3 times each week for 8 weeks, which resulted in a much higher cumulative dose and a total of eight deaths (Meregalli et al. 2012). Major differences in animal models like these make it difficult to compare results conclusively.

Similar to the rat models, although fewer studies have been completed in mice, there is no standard mouse model. Studies have been completed to try to develop one, yet there is little consistency across these attempts. One study used 2.5-month-old Swiss OF1 female mice and administered 1 mg/kg bortezomib by subcutaneous injection twice each week for 6 weeks; mild to moderate sensory neuropathy was reported (Bruna et al. 2010). Another more recent study attempted to evoke bortezomib-induced neuropathy using a single dose (0.2 mg/kg, 0.5 mg/kg, 1 mg/kg) by intraperitoneal injection in C57BL/6 mice and found a dose-dependent effect lasting up to 11 days after administration (Trevisan et al. 2013). It is understandable that there is variation in experimental design during development of a successful mouse model of bortezomib-induced neuropathy, but once an accurate model is established, it is important for that model to be standardized and used in all bortezomib studies.

Others

Other chemotherapy agents that result in neuropathy include thalidomide and suramin. Thalidomide induces neuropathy in roughly one-third of patients, and suramin is linked to a dose-related peripheral neuropathy. There are no established animal models of neuropathy induced by either of these substances because of the minimal in vivo studies that have been completed (Kirchmair et al. 2007; Russell et al. 2001). Because these drugs are commonly used to treat different cancers, it is important for effective animal models to be established and standardized for further study.

Evaluation of Peripheral Neuropathy in Rodent Models of CIPN

The methods of evaluation of neuropathy used are disparate across every group of drugs causing CIPN. It is not uncommon for studies to evaluate the degree of neuropathy using a series of behavioral tests or electrophysiologic methods only rather than a combination of the two; such studies can be found in every type of CIPN (Boyle et al. 1996; Mo et al. 2012; Nodera et al. 2011; Saika et al. 2009; Ta et al. 2013; Zheng et al. 2012). Investigations completing electrophysiologic testing also lack consistent scheduling for the testing in all groups of drugs. All studies conduct testing at baseline, yet the schedule following treatment varies. Some studies will record data immediately upon reaching the cumulative dose, some will wait a certain number of days after treatment, and some will record data upon reaching the cumulative dose and conduct follow-up testing weeks later (Carozzi

et al. 2009; Cavaletti et al. 2007; Meregalli et al. 2012; Nodera et al. 2011; Verdu et al. 1999).

To date, there are no standard histologic or pathologic analyses completed for studies of CIPN. There is a history of great variations in this type of evaluation, with some studies conducting several histologic examinations, including nerve cross-sectional area, axonal counts, myelin density, g ratio, and average intraepidermal nerve fiber density (Cavaletti et al. 2001; Melli et al. 2006). There are, however, studies that do not conduct any type of histopathologic analyses (Nodera et al. 2011; Nozaki-Taguchi et al. 2001; Ta et al. 2013). Clearly, some investigators find these experiments necessary, whereas others do not. Another issue is with the studies that have completed these evaluations. Similar to behavioral and electrophysiologic testing, no specific set of analyses is completed; the analyses vary greatly not only in what tests are being done but also in what tissues.

Summary and Conclusions

To date, many studies modeling peripheral neuropathy caused by different chemotherapy agents have been carried out in rodents. Although many of these studies mimic peripheral neuropathy seen in patients, at least in some aspects, the field lacks consistency in the models used because of a variety of reasons. It is difficult to determine a maximum dose of chemotherapy that can be tolerated in animals when the experiments being completed are so vastly different. It is necessary for these studies to be standardized, so all studies will be done using the same animal of a specific genetic background, with animals administered the same dose over the same period of time. Investigators also need to take the time to arrange for laboratory members to be trained properly so that a switch can be made in the mode of administration from intraperitoneal injection to intravenous injection. Although intravenous injections are more difficult to do, it is more appropriate to model the mode of drug delivery based on what is used in patients. Finally, the best combination of outcome measurements needs to be determined, whether these measurements are strictly behavioral, electrophysiologic, histopathologic, or a specific combination. A major shortcoming of the behavioral tests is that they are all evoked responses, whereas the majority of patients complain of spontaneous painful paresthesias that are difficult to evaluate in animals. Electrophysiologic studies are useful when the chemotherapeutic drug causes degeneration of myelinated fibers but are insensitive to degeneration of unmyelinated axons seen in early stages of CIPN. Similarly, nerve morphometry using light microscopy fails to evaluate unmyelinated axon degeneration. Although electron microscopy can be carried out to quantify degeneration of unmyelinated axons, it is seldom done. Quantitation of intraepidermal nerve fiber density is becoming a common, more objective tool for evaluation of peripheral neuropathy both in patients and in animal models of other peripheral neuropathies. Because it evaluates the distal-most portions of the axons, it is a

sensitive tool to evaluate most neuropathies that are often length dependent (i.e., degeneration that starts in the distal-most portion of longest axons first).

To move the field forward and make comparisons of data from different laboratories more valid, we advocate for a consensus on choice of animals (similar sex, age, and genetic background), mode and schedule of drug delivery, and outcome measures used in evaluation. This issue is critically important as CIPN becomes a significant clinical target for development of potential neuroprotective drugs.

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