COMPARISON OF NEUROPATHY-INDUCING EFFECTS OF ERIBULIN MESYLATE, PACLITAXEL AND IXABEPILONE IN MICE

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Running Title: Neuropathy induced by Eribulin, Paclitaxel, and Ixabepilone

Keywords: eribulin mesylate, paclitaxel, ixabepilone, microtubules, chemotherapy-induced neuropathy,
Chemotherapy-induced neurotoxicity is a significant problem associated with successful treatment of many cancers. Tubulin is a well-established target of antineoplastic therapy, however tubulin targeting agents, such as paclitaxel and the newer epothilones, induce significant neurotoxicity. Eribulin mesylate, a novel microtubule targeting analog of the marine natural product halichondrin B recently demonstrated antineoplastic activity in metastatic breast cancer patients, with relatively low incidence and severity of neuropathy. The mechanism of chemotherapy-induced neuropathy is not well understood. One of the main underlying reasons is incomplete characterization of pathology of peripheral nerves from treated subjects, either from patients or pre-clinically from animals. The current study was conducted to directly compare in mice the neuropathy inducing propensity of three drugs: paclitaxel, ixabepilone and eribulin mesylate. Because the drugs have different potencies and pharmacokinetics, we compared them based on a maximum tolerated dose (MTD) basis. Effects of each on caudal and digital nerve conduction velocity, nerve amplitude, and sciatic nerve and dorsal root ganglion morphology at 0.25xMTD, 0.5xMTD, 0.75xMTD and MTD were compared. Paclitaxel and ixabepilone at their respective MTDs produced significant deficits in caudal nerve conduction velocity, caudal amplitude and digital nerve amplitudes, as well as moderate to severe degenerative pathological changes in dorsal root ganglia and sciatic nerve. In contrast, eribulin mesylate produced no significant deleterious effects on any nerve conduction parameter measured, and caused milder, less frequent effects on morphology. Overall, our findings indicate that eribulin
mesylate induces less neuropathy in mice than paclitaxel or ixabepilone at equivalent MTD-based doses.

**Introduction**

Peripheral neuropathy is a common dose-limiting toxicity of many chemotherapies. Chemotherapy-induced peripheral neuropathy (CIPN) causes numerous debilitating symptoms, impairs functional capacity, and results in dose reductions or possible cessation of chemotherapy. As a consequence, effective chemotherapies with a lower propensity to induce neuropathy would be favored.

Breast cancer is the most common cause of cancer-related death among women in the USA. Many patients progress from early stage breast cancer to metastatic disease within short periods of time. While several chemotherapeutic agents exist for metastatic disease, overall prognosis remains poor with the 5 year survival rate approximating only 23% (1).

Paclitaxel is often administered as first-line therapy to breast cancer patients with metastatic disease, achieving overall response rates in the range of 30%-60%, or 20-40% when used as second-line or salvage therapy (2). Taxanes are among the most effective antineoplastics against many cancers, but peripheral neurotoxicity has limited their use (3, 4). Chronic neuropathic pain affects between 20 and 50% of women after their breast cancer treatment (5). Another chemotherapeutic administered to metastatic breast cancer patients whose cancer is resistant or no longer responding to paclitaxel is the recently approved ixabepilone, which is a semi-synthetic analogue of epothilone B with antineoplastic activity against taxane resistant cell lines (6, 7, 8). While ixabepilone may be useful for treatment of locally advanced or metastatic breast cancer, it induces
neuropathy in up to 72% of patients (9). Both ixabepilone and paclitaxel are microtubule-stabilizing agents that promote polymerization of microtubules (10). However, the precise mechanisms of paclitaxel and ixabepilone induced neurotoxicity are not yet completely understood (11-13). One of the reasons for this is the incomplete characterization of function and pathology of peripheral nerves from chemotherapy-treated subjects, either from patients or preclinically from animals. Further studies in this area are thus warranted.

Eribulin mesylate (previously E7389), another promising microtubule-targeting agent, is currently under FDA fast-track review. Recently, positive results from randomized Phase II and III trials of eribulin mesylate in advanced metastatic breast cancer have been reported (14,15). Eribulin mesylate is a structurally simplified macrocyclic ketone analog of halichondrin B, and inhibits microtubule dynamics via a novel mechanism relative to other tubulin-targeting agents including the taxanes, vinca alkaloids and epothilones. It binds specifically and with high affinity to microtubule plus ends thereby suppressing microtubule dynamics (16). Preclinically, eribulin mesylate has shown potent anti-cancer activity in vitro and in vivo (17). Preliminary reports from clinical observations with eribulin mesylate suggest that at efficacious exposures there is relatively low incidence and severity of neuropathy (18). On this basis, preclinical studies were performed to directly compare MTD regimens (and equivalent fractions thereof) of paclitaxel, eribulin mesylate and ixabepilone for neuropathy induction in mice, based on nerve conduction velocity (NCV), amplitude, and sciatic nerve and dorsal root ganglia (DRG) morphology endpoints. We describe herein our findings.
Materials and Methods

Test Materials

The following chemicals were used in this study: Eribulin mesylate (synthesized at Eisai Research Institute) was stored at -80°C in the dark. Paclitaxel (C_{47}H_{51}NO_{14}) was purchased from LC Laboratories, Woburn, MA and stored until ready for use at -20°C in the dark. Ixabepilone (C_{27}H_{42}N_{2}O_{5}S) was purchased from Myoderm Medical, NJ and stored at 4°C in the dark.

Formulations

Eribulin mesylate was dissolved in 100% anhydrous DMSO (Sigma-Aldrich, St. Louis, MO, Cat. No. D2650) to produce a 10 mg/ml stock solution which was separated into aliquots and stored in the dark at -80°C until day of administration. On each administration day the stock solution was thawed and diluted with saline to a final concentration of 0.25 mg/mL in 2.5% DMSO/97.5% saline yielding dosing solutions in a 10 mL/kg volume.

Paclitaxel was dissolved in ethanol (100%) at 10% of final desired volume and vortexed for 2-3 min. An equal volume of cremophor (10% of final volume) was then added and the mixture re-vortexed for about 10 min. Immediately prior to injection, ice cold saline was added to final volume (as 80% of final) and the solution was maintained on ice during dosing. Dosing solutions were made fresh daily and dosed in an administration volume of 10 mL/kg.

Ixabepilone was purchased as part of an IXEMPRA kit for clinical administration.
The ixabepilone solution was prepared as described in the package insert. Basically, the kit consists of two vials, one containing 47 mg ixabepilone powder and the other 23.5 mL diluent, which were stored in the refrigerator at 4°C. The total volume of diluent was added to the total amount of powder, so after constituting the concentration of ixabepilone in the solution was 2 mg/mL. (The diluent supplied consists of a sterile non-pyrogenic solution of 52.8% (w/v) purified polyoxyethylated castor oil and 39.8% (w/v) dehydrated alcohol). The formulated ixabepilone stock solution (2 mg/mL) was immediately aliquoted and stored at -80°C until use. On each experimental day the stock solution was diluted by adding 50% ethanol/50% cremophor and subsequent vortexing to yield a resultant solution that was five times the required dosing concentration. Finally, 4x volumes of PBS were added while vortexing, to achieve final dosing concentration in 10 mL/kg.

**Animals**

Female BALB/c mice (approximately 7-8 weeks old at onset of dosing) were used for all experiments. Mice were obtained from Harlan Laboratories Inc (Indianapolis, IN) and maintained with ad libitum access to water and a standardized synthetic diet (Harlan Teklab), both prior to and during the study. Animal housing and procedure room temperature and humidity were maintained at 20 ± 2°C and 55 ± 10% respectively. Artificial lighting provided a 12 h light/12 h dark cycle (light 7am-7pm). All experimental protocols were approved by the Institutional Animal Care and Use Committee of Eisai, Baltimore and adhered to all of the applicable institutional and governmental guidelines for the humane treatment of laboratory animals.
MTD Determination

For each experimental compound, a maximum tolerated dose (MTD) on a \([Q2D \times 3]\) x 2 weeks schedule (every other day for 3 injections with a two-day rest between weekly cycles for a total of 6 injections) was determined using groups of 10 mice each. MTD was defined as the highest dose level in which no more than 10% deaths occurred and/or at which no mice displayed >20% individual weight loss and/or overt clinical signs of distress and/or inability to eat and drink, requiring euthanasia.

Doses chosen for the MTD studies were based upon antitumor activity and/or neuropathy-inducing doses in mice as previously described (12,17,19 and 20). Doses used for eribulin mesylate were 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75 mg/kg per dose administration. Doses used for paclitaxel were 20, 25, 30, 35, 40 and 45 mg/kg per dose administration. Doses used for ixabepilone were 2.0, 2.25, 2.5, 2.75, 3.0, 3.5 and 4.0 mg/kg per dose administration. All injections were made into the caudal vein at a volume of 10 mL/kg.

Mice were weighed three times a week during the 2 week treatment period and for two weeks following completion of dosing. Day 1 refers to the first administration of experimental compound or vehicle. Day 12 refers to the day when all six doses of chemotherapeutic/vehicle had been administered. Day 26 refers to the final experimental day (two weeks after completion of dosing). Clinical signs and survival were monitored daily.

Nerve conduction velocity and amplitude measurement

Baseline NCV was measured one week prior to initiation of dosing in 50 mice.
Mice were subsequently randomized into the 5 treatment groups such that mean digital NCVs (as well as all other parameters) for each group of 10 mice were equivalent. Each group then received vehicle or chemotherapy (at MTD, 0.75xMTD, 0.5xMTD or 0.25xMTD) on a [Q2D x 3] x 2 week schedule. Post treatment nerve conduction measurements were made 24h after last chemotherapy dose.

During all recording sessions, mice were anesthetized with 2% isoflurane (by inhalation, for induction and maintenance) and placed in a prone position. Throughout testing, animals were positioned on a warm heating pad with rectal temperature monitored and maintained between 37.0 - 41.0°C. Platinum subdermal needle electrodes (Grass Technologies, West Warwick, RI) were used for both recording and stimulation. NCV and the peak compound action potential amplitude were assessed in both caudal and digital nerves. Caudal and digital NCV were recorded orthodromically with recording sites at the proximal tail and the lateral malleolus, respectively. Supramaximal stimulation was achieved using a constant voltage square wave pulse (0.02 to 0.05 msec duration) isolated from ground produced by the MP100 (BIOPAC Systems Inc., Santa Barbara, CA, USA). Each nerve segment stimulation was repeated a total of at least 3 times, up to a maximum of 6 times, with increasing voltage until the maximal response had been achieved, as evidenced by no further increase or a reduction in amplitude despite increase in voltage. Neuroelectrical signals were impedance matched and differentially amplified with a gain of 20,000 and a frequency band of 20 Hz to 3 kHz using a BIOPAC MP100 and AcqKnowledge software version 3.7.3 (BIOPAC Systems Inc.). Latencies were scored from stimulus onset, and amplitudes from baseline, using computer cursors. Latency measurements were scored to the nearest 0.01 msec and
amplitude measurements were scored to the nearest 0.01 µV.

Differences in measurements across groups were determined by direct statistical comparisons (ANOVA, followed by Tukey’s post-hoc comparisons; using Prism Graphpad Software Version: 4.03) of the amplitude and conduction velocity data. Statistical significance was defined as p<0.05.

Caudal NCV was recorded from electrodes in a bipolar configuration at the base of the tail (at the hair line); the stimulating cathode being positioned 35 mm further distal. Digital NCV was recorded using stimulation at the base of the second toe and recording at the level of the lateral malleolus. The distance travelled was measured for each mouse and is generally between 9-14 mm. The response latency at supramaximal stimulation divided by the distance between the electrodes used for recording and stimulating is a measure of the conduction velocity in the nerve segment under investigation. Amplitudes measure the baseline to peak amplitude of the neural response.

**Sciatic nerve and dorsal root ganglia histology**

Immediately after recording of final nerve conduction parameters, 5 randomly selected mice from each group were chosen for nerve/DRG excision. Sciatic nerve segments were dissected from the region immediately before the trifurcation of the sciatic nerve (into the common peroneal, tibial and sural branches); L4 and L5 DRG were dissected from the spinal cord. Samples were then fixed in 3% glutaraldehyde and 4% paraformaldehyde/2% glutaraldehyde, respectively. Samples were processed and resin-embedded according to previously published protocols (21-24) and used for light microscope determinations. After embedding, samples were cut in 1 µm semi-thin
sections with a microtome RM2265 (Leica Microsystems GmbH, Wetzlar, Germany) and sections stained with toluidine blue for light microscopy with a Nikon Eclipse E200 light microscope (Nikon, Florence, Italy). Section codes were masked before examination so that two independent examiners were unaware of the treatment administered to the examined animal.

**Results**

**Eribulin Mesylate**

As expected in this MTD-finding experiment, mice treated with 1.5 mg/kg and 1.75 mg/kg eribulin mesylate on a [Q2D x 3] x 2 week schedule displayed significant body weight losses averaging between 14-17% (Fig. 1A) as well as clinical signs characterized by piloerection, general unkempt condition and decreased motor activities. Average weight losses in groups treated with eribulin mesylate at 1.25 mg/kg or lower were minimal (<10%) and mice in these groups displayed no overt clinical signs. Weight loss was completely recovered following cessation of dosing. The MTD of eribulin mesylate was determined to be 1.75 mg/kg when administered according to this regimen, with an average group weight loss of 17%.

After two weeks of dosing with eribulin mesylate, there were no observed deficits in either caudal or digital NCV or amplitude (Fig. 2). Surprisingly, dosing eribulin mesylate at MTD (1.75 mg/kg) produced a significant increase in caudal amplitude compared to control, and a similar trend was noted for 0.75xMTD (1.31 mg/kg). A possible explanation for this paradoxical finding may be due to dehydration of the tail in these high dose groups. Since the amplitude of the response to nerve stimulation is a measure of the density of the responding fibers, decreased tissue volume secondary to
dehydration may present as an artifactual increase in amplitude. However, since high
dose paclitaxel- and ixabepilone-treated mice also exhibited similar weight loss without
accompanying augmentation of caudal amplitude, this may not be the only explanation of
this finding. The underlying reason for this observation will require further study.

A dose-dependent effect on sciatic nerve morphology was evident with 0.5xMTD
(0.875 mg/kg) to MTD (1.75 mg/kg) doses of eribulin mesylate. At these doses, eribulin
mesylate induced mild to moderate pathological changes consistent with axonal
degeneration (Fig. 5B). Axonal degeneration at MTD seemed to affect both large- and
small- diameter fibers; no regenerative figures (e.g. thin myelinated fibers) were
present. Clear cytoplasmic vacuolation of DRG neurons appeared at 0.75xMTD to MTD
eribulin mesylate doses, while dark inclusions were only very rarely observed at the
MTD dose (Fig. 6B).

**Paclitaxel**

As shown in Fig. 1B, all mice receiving more than 30 mg/kg paclitaxel on a [Q2D
x 3] x 2 week schedule suffered appreciable weight loss (>20% of original starting
weight). Mice in these groups also displayed significant hind-limb nerve malfunction;
these animals were sacrificed before receiving all planned doses in the study. The
remaining mice in the 35 mg/kg group (2/10) recovered their weight loss after dosing. In
contrast, doses of 30 mg/kg paclitaxel or less were well tolerated, with mice in these
groups receiving the entire chemotherapy regimen. Average body weights per treatment
group over the duration of the treatment regimen are shown as a percentage of starting
weight (Day 1) in Fig. 1B. MTD of paclitaxel was determined to be 30 mg/kg when
administered according to this regimen.

After two weeks of dosing with paclitaxel, clear dose dependent decreases in caudal NCV and amplitude were evident (Fig. 3). The MTD dose of paclitaxel (30 mg/kg) caused significant reduction in caudal NCV compared to control (p<0.01) while MTD and 0.75xMTD doses (30 and 22.5 mg/kg, respectively) caused significant reduction in caudal amplitude compared to control (p<0.001). Paclitaxel doses of 0.5xMTD and below had no effect on caudal NCV and amplitude. MTD and 0.75xMTD doses of paclitaxel (30 mg/kg and 22.5 mg/kg, respectively) also caused significant reductions in digital nerve amplitude (Fig. 3D; p<0.01 and p<0.05 respectively). In contrast, although all paclitaxel doses tested tended to adversely affect digital NCV, no dose effect attained statistical significance (Fig. 3C).

A dose-dependent effect of paclitaxel on sciatic nerve morphology was observed. At MTD (30 mg/kg), paclitaxel caused severe pathological changes consistent with axonal degeneration (Fig. 5C). Axonal degeneration seemed to affect both large- and small- diameter fibers, and no regenerative figures (e.g. thin myelinated fibers) were present. The severity of axonopathy was milder but still clearly evident in groups treated with 0.75xMTD (22.5 mg/kg) or 0.5xMTD (15.0 mg/kg) paclitaxel (Fig.1S, A and B supplemental data). In DRG, dose-dependent adverse effects of paclitaxel were also evident. Paclitaxel at 0.5xMTD (15 mg/kg, Fig 2S, A supplemental data) caused formation of dark inclusions in the cytoplasm. DRG from the 0.75xMTD (22.5 mg/kg) group also had clear vacuolations in the cytoplasm of neurons and also in satellite cells (Fig. 2S, B supplemental data). At MTD paclitaxel (30 mg/kg), a proportion of neurons in the DRG were degenerating and their cytoplasm appeared much darker than normal
neurons (Fig. 6C). Dose dependent axonal degeneration was present in the proximal axons at 0.5xMTD and above (Fig 6C and 2S, A supplemental data).

**Ixabepilone**

All mice receiving ixabepilone doses over 3.0 mg/kg on a [Q2D x 3] x 2 week schedule suffered appreciable weight loss (over 20% of original weight) and displayed overt clinical signs of non-well-being (including piloerection and unkempt coats); these animals were euthanized prior to the end of the planned doses. Moderate weight losses (between 11 and 15%) were observed in the middle dose groups (2.75 and 3.0 mg/kg ixabepilone), which reversed during the 14 day recovery/observation period after cessation of dosing. In contrast, 2.5 mg/kg or lower of ixabepilone caused minimal weight loss (=/<10%), no overt clinical signs, and was well tolerated by the mice. Minimal clinical signs were observed in the 2.75 and 3.0 mg/kg dose groups. The MTD of ixabepilone on a [Q2D x 3] x 2 week schedule was determined to be 3 mg/kg (Fig. 1C).

After two weeks of dosing with ixabepilone, a clear dose response for decreasing caudal NCV and amplitude was evident (Figs. 4A and 4B). Doses of 0.5xMTD to MTD (1.5 to 3 mg/kg) caused significant reductions in caudal NCV, while doses of 0.75xMTD to MTD (2.25 to 3 mg/kg) caused significant reduction in caudal amplitude. At MTD (3.0 mg/kg), ixabepilone significantly reduced both digital NCV and amplitude (Figs. 4C and 4D).

Ixabepilone at doses below MTD (0.75 mg/kg to 2.22 mg/kg), generally had minimal effect on morphology of nerve fibers, although rare and sporadic alterations were present in sciatic nerves (Fig1S, C supplemental data). Sciatic nerves from mice...
receiving MTD ixabepilone (3 mg/kg) had severe and frequent pathological changes, represented by different stages of axonal degeneration. As shown in Fig. 5D, axonal degeneration seemed to affect both small- and large- diameter fibers and no regenerative figures (e.g. thin myelinated fibers) were present. Ixabepilone at its MTD caused very severe, frequent morphological alterations both in neuronal and glial compartments (Fig. 6D) in the DRG. The neurons had dark cytoplasmic inclusions, often localized to the perinuclear area. Moreover, clear vacuolations and swelling phenomena were evident in the cytoplasm of satellite cells. Changes were indicative of severely injured and degenerating cells. Adverse effects of ixabepilone administration were also evident with other doses. DRG from mice receiving ixabepilone at 0.25xMTD and 0.75xMTD displayed occasional cytoplasmic swelling in the satellite cells (Fig. 2S, C and D supplemental data). Cytoplasmic dark inclusions and degenerating neurons, as well as vacuoles of satellite cells were also evident in the 0.75xMTD and MTD ixabepilone groups (Fig. 6D x and Fig 2S, D supplemental data). Moreover, rare episodes of clear cytoplasmic vacuolation of neurons were also observed.

Discussion

Chemotherapy-induced peripheral neurotoxicity is a major clinical problem representing the dose-limiting side-effect of many antineoplastic drugs, having a significant negative impact on quality of life and often resulting in treatment delays or discontinuation (25-27). Indeed, neurotoxic side effects associated with chemotherapies are second in frequency only to hematological toxicities. Unlike the hematological side
effects for which there are known effective treatments, neurotoxicity side effects cannot be effectively treated or prevented (28,29). Neurotoxicity may develop as a consequence of treatment with platinum analogs (cisplatin, oxaliplatin, carboplatin), taxanes (paclitaxel, docetaxel), vinca alkaloids (vincristine) and, more recently, thalidomide and bortezomib as well as epothilones (9, 30). The degree and type of neuropathy depends on the chemotherapy drug, dose intensity and cumulative dose. Recovery from peripheral neurotoxicity symptoms is often incomplete and a long period of regeneration is sometimes required to restore function.

Microtubule targeting agents were first introduced into clinical oncology in the 1960s and are essential components in the therapy of many cancers, including lymphoma as well as breast, ovarian, lung, and head and neck cancers (31). Despite their associated neurotoxic side-effects, these agents in general remain the most effective treatment options for survival prolongation in advanced disease (32). The anticancer effects of tubulin-targeting agents generally derive from their ability to bind to microtubules, interfere with mitotic spindle formation, and ultimately to block mitosis, resulting in cell death. However, since somatic neurons do not divide, the neurotoxic effects of tubulin-targeting agents could derive from effects on interphase microtubules not involved in mitosis. Although some information exists, our understanding of the mechanisms behind the neurotoxic effects of tubulin-targeting agents is far from complete. Further studies of the neurotoxic side effects of tubulin targeting chemotherapies are thus warranted (33).

For the past 20 years, several chemotherapy-induced neuropathy animal models have been published, most commonly using rodents. Similar to the human situation, chemotherapy-induced neuropathy rodent models are characterized by
neurophysiological deficits as well as morphological alterations in DRG and myelinated nerve fibers (23, 34-36). Somewhat surprisingly, despite the existence of such models, to our knowledge there has never been a systematic comparison of the various microtubule targeting agents in preclinical models.

Eribulin mesylate, a non-taxane microtubule dynamics inhibitor belonging to the halichondrin class of antineoplastic agents, is a structurally-simplified macrocyclic ketone analogue of halichondrin B, a natural product originally isolated from the marine sponge *Halichondria okadai* (17). Eribulin mesylate is currently in development for the treatment of advanced breast cancer and other solid tumors. Other microtubule-targeting agents used to treat breast cancer patients, including paclitaxel and ixabepilone, display a common dose-limiting toxicity of peripheral neuropathy. The purpose of this study was to investigate neuropathy-inducing effects of eribulin mesylate in mice compared to paclitaxel or ixabepilone.

All three chemotherapeutic drugs have anti-neoplastic activity in various animal models (6, 7, 17) and human cancer conditions (2, 37, 38). Importantly, all three compounds have demonstrated efficacy in multiple in vivo cancer efficacy models at doses often below the MTD described in this study in the case of eribulin mesylate (17), or generally comparable to the MTD, in the case of paclitaxel (17) and ixabepilone (6,7). For example, eribulin has shown efficacy in a human breast [MDA-MB-435] cancer xenograft model in athymic mice at 0.25 to 1.0 mg/kg using a Q2D×3 [×4 weeks] schedule (17). These efficacious doses are below the MTD described in this study (1.75mg/kg). Paclitaxel has shown antineoplastic effects in the same breast xenograft model at 25 mg/kg, similar to the MTD described in this study (30 mg/kg) (17).
Ixabepilone has shown antitumor activity in mouse models including breast [KPL4,Pat-21], at a dose range of 3.2 to 6.0 mg/kg administered Q2Dx5 or Q4Dx3 (6,7), which is also similar to the ixabepilone MTD (3 mg/kg) in this study. Therefore, it is reasonable to assume that the mice used in this study received similar (paclitaxel, ixabepilone) or greater (eribulin mesylate) exposures than those required for efficacy.

Because the drugs have different potencies and pharmacokinetics, we compared them based on a MTD basis using a neuropathy-inducing dosing regimen, [Q2D x3] for 2 weeks. Once MTD doses were defined as 1.75 mg/kg eribulin mesylate, 30 mg/kg paclitaxel, and 3.0 mg/kg ixabepilone, we administered 0.25, 0.5xMTD, 0.75xMTD and MTD doses and evaluated their effects on NCV, amplitude, and morphology of sciatic nerves and DRG. Using these comparisons, ixabepilone and paclitaxel dosed on a [Q2D x3] for 2 week schedule were found to produce significant deficits in conduction parameters of caudal and digital nerves at 0.5xMTD doses and above. In contrast, eribulin mesylate induced no deficits in any monitored nerve conduction parameter (velocity or amplitude) at any dose tested, including MTD (1.75 mg/kg). Similarly, although all compounds produced morphological changes in DRG and sciatic nerves at MTD, the changes induced by eribulin mesylate were noticeably fewer and less severe than those seen with paclitaxel or ixabepilone at their respective MTDs. Since all three agents target tubulin, the question arises as to why eribulin mesylate is less damaging to neurons in this mouse model.

Paclitaxel and ixabepilone are considered microtubule stabilizing agents, since they promote polymerization of microtubules and increase polymer mass in cells. In contrast, eribulin mesylate is a microtubule-destabilizing agent since it binds to the vinca-
binding domain of tubulin and inhibits tubulin polymerization, thereby inducing cell cycle arrest and apoptosis (10, 39). At low concentrations both stabilizers and destabilizers suppress microtubule dynamics without changing polymer mass (40, 41). As the understanding of the interactions of tubulin targeting agents at the molecular level increases, differences in activity and side-effects may become clearer. Differences in microtubule-binding properties may have significant effects on the toxicity profile of each agent (10, 36).

In this context, Jordan and colleagues found that eribulin mesylate affects microtubule growth phases as opposed to the shortening phase, in association with sequestration of tubulin into aggregates (42). Eribulin mesylate binding along microtubule sides is at least 10 fold lower affinity than at the plus ends (16). This is in contrast to the binding pattern of paclitaxel and ixabepilone, which each affect both the growth and shortening phases of microtubule dynamics, and each of which are thought to target the same, or near the same, binding site on β-tubulin (10, 43). As neuronal microtubules appear responsible for intracellular transport of essential molecules along the axon, it is possible that binding along the microtubule sides may inhibit transport of essential molecules with resulting peripheral neurotoxicity (44). These theories require further investigation.

Despite the limitations of this study, such as its relatively short duration and small sample size, the findings suggest that if these animal studies are corroborated in the clinic, eribulin mesylate could have a positive impact on the side effect profile of cancer patients receiving microtubule-based therapies.
REFERENCES


FIGURE LEGENDS

Figure 1: Body weight of mice. Body weight of mice expressed as a percentage of their starting weight following treatment with vehicle vs. eribulin mesylate (A), paclitaxel (B), and ixabepilone (C) on a [Q2D x 3] for 2 week schedule.

Figure 2: Eribulin mesylate effect on NCV and amplitude. Eribulin mesylate at 0.25, 0.5, 0.75 and MTD doses had no inhibitory effect on caudal nerve conduction velocity (A). Eribulin at MTD increased caudal amplitude with no significant effects at other doses (B). Eribulin did not affect digital nerve conduction or amplitude at any dose (C and D). [Figure depicts mean ± sem]

Figure 3: Paclitaxel effect on NCV and amplitude. Paclitaxel at its MTD dose reduced caudal NCV (A), reduced caudal amplitude (B). Paclitaxel had no significant affect on digital nerve conduction (C). Paclitaxel at its MTD and 0.75 MTD doses reduced digital amplitude (D). [Figure depicts mean ± sem]

Figure 4: Ixabepilone effect on NCV and amplitude. Ixabepilone at 0.5, 0.75 and MTD doses decreased caudal NCV (A). Ixabepilone at 0.75 and MTD doses produced dose dependent decreases in caudal nerve amplitude (B). Ixabepilone at MTD caused a significant reduction in digital nerve conduction velocity (C) and amplitude (D). [Figure depicts mean ± sem]
Figure 5: Effect of MTD doses of eribulin mesylate, paclitaxel and ixabepilone on sciatic nerve morphology. Morphological changes were most severe with paclitaxel (C) and ixabepilone (D) with both agents causing frequent and severe pathological changes consistent with axonal degeneration of both large and small fibers highlighted with arrows and arrowheads respectively. No regenerative figures (e.g., thin myelinated fibers) were evident with either treatment. Eribulin mesylate at its MTD dose also induced some mild pathology (B), but it was less frequent than with paclitaxel or ixabepilone, more closely resembling vehicle-treated mice (A). [Scale: bar =20 microns]

Figure 6: Effect of MTD doses of eribulin mesylate, paclitaxel and ixabepilone on DRG morphology. DRG morphology at the light microscope level showed changes after each chemotherapy, at its MTD. Ixabepilone (D) caused most severe and frequent morphological changes both in neuronal and glial compartments. Severely injured, degenerating neurons are outlined (red panel). Dark cytoplasmic inclusions were evident, often localized in the perinuclear area. Vacuolations (white arrows) and swelling phenomena (red arrowheads) were evident in the cytoplasm of satellite cells (D). DRG from paclitaxel-treated mice (C) also displayed degenerating nerve cells (outlined by red circle) with dark cytoplasm (red arrows) and clear vacuolations in cytoplasm of satellite cells (white arrow). Alterations in the proximal axons of DRG were also observed (white circle). DRG from eribulin mesylate-treated mice (B) showed mild pathological changes evidenced by some cytoplasmic vacuolation (white arrow) and degenerating nerve cells (red arrow). [Scale: bar =20 microns]
Figure 1A
Figure 1B
Figure 1C
Figure 2

A. Caudal nerve conduction velocity

B. Caudal nerve amplitude

C. Digital nerve conduction velocity

D. Digital nerve amplitude

*p<0.05 vs vehicle"
Figure 3
Figure 4
Figure 6:
## COMPARISON OF NEUROPATHY-INDUCING EFFECTS OF ERIBULIN MESYLATE, PACLITAXEL AND IXABEPILONE IN MICE


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