Comparison of Neuropathy-Inducing Effects of Eribulin Mesylate, Paclitaxel, and Ixabepilone in Mice

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Abstract

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 analogue of the marine natural product halichondrin B, has recently shown antineoplastic activity, with relatively low incidence and severity of neuropathy, in metastatic breast cancer patients. 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 preclinically 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 these drugs have different potencies and pharmacokinetics, we compared them on the basis of a maximum tolerated dose (MTD). Effects of each drug on caudal and digital nerve conduction velocity, nerve amplitude, and sciatic nerve and dorsal root ganglion morphology at 0.25 × MTD, 0.5 × MTD, 0.75 × MTD, 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 pathologic 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. Cancer Res; 71(11); 1–11. ©2011 AACR.

Introduction

Peripheral neuropathy is a common dose-limiting toxicity of many chemotherapeutic regimens. 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 chemotherapeutic regimens 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 Unites States. Many patients progress from early-stage breast cancer to metastatic disease within short periods of time. Although several chemotherapeutic agents exist for metastatic disease, the overall prognosis remains poor, with the 5-year survival rate approximating to only 23% (1).

Paclitaxel is often administered as first-line therapy in breast cancer patients with metastatic disease, achieving overall response rates in the range of 30% to 60%, or 20% to 40% when used as second-line or salvage therapy (2). Taxanes are among the most effective antineoplastic agents against many cancers, but their side effect of 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 agent administered to metastatic breast cancer patients whose cancer is resistant or no longer responding to paclitaxel is the recently approved ixabepilone, which is a semisynthetic analogue of epothilone B with antineoplastic activity against taxane-resistant cell lines (6–8). Although ixabepilone may be useful for the 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 neurotoxicities are not yet completely understood (11–13). One of the reasons for this is the incomplete characterization of function and pathology of peripheral nerves in 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 undergoing Food and Drug Administration (FDA) fast-track review. Recently, positive results from randomized phase II and III trials of eribulin mesylate in patients with advanced metastatic breast cancer have been reported (14, 15). Eribulin mesylate is a structurally simplified, macrocyclic, ketone analogue of halichondrin B, and it inhibits microtubule dynamics through a novel mechanism relative to other tubulin-targeting agents, including the taxanes, \textit{Vinca} alkaloids, and epothilones. It binds with high affinity and specificity to positively charged ends of microtubules, thereby suppressing microtubule dynamics (16). Preclinically, eribulin mesylate has shown potent anticancer activity \textit{in vitro} and \textit{in vivo} (17). Preliminary reports from clinical observations of therapy with eribulin mesylate suggest that, at efficacious exposures, there is relatively low incidence and severity of neuropathy (18). Based on this finding, preclinical studies were conducted to directly compare maximum tolerated dose (MTD) regimens (and equivalent fractions thereof) of paclitaxel, eribulin mesylate, and ixabepilone for neuropathy induction in mice, which was evaluated by parameters including nerve conduction velocity (NCV), amplitude, and sciatic nerve and dorsal root ganglia (DRG) morphologic endpoints. In this article, we describe our findings from this study.

Materials and Methods

Test materials

The following chemicals were used in this study: eribulin mesylate (synthesized at Eisai Research Institute, Andover, MA) was stored at \(-80^\circ\text{C}\) in the dark. Paclitaxel (C\(_{47}\)H\(_{51}\)NO\(_{14}\)) was purchased from LC Laboratories and stored at \(-20^\circ\text{C}\) in the dark until required for use. Ixabepilone (C\(_{27}\)H\(_{42}\)N\(_{2}\)O\(_{5}\)S) was purchased from Myoderm Medical and stored at 4°C in the dark.

Formulations

Eribulin mesylate was dissolved in 100% anhydrous dimethyl sulfoxide (DMSO; Sigma-Aldrich; catalogue no. D2650) to produce a 10-mg/mL stock solution, which was separated into aliquots and stored in the dark at \(-80^\circ\text{C}\) until the day of administration. On each day of administration, 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 to yield dosing solutions in a 10-mL/kg volume.

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

Ixabepilone was purchased as part of an IXEMPRA kit (Bristol-Myers Squibb) for clinical administration. The ixabepilone solution was prepared according to instructions provided in the package insert. Basically, the kit consists of 2 vials, 1 containing 47 mg ixabepilone powder and the other containing 23.5 mL diluent, which were stored in the refrigerator at 4°C. The total volume of the diluent was added to the total amount of powder; therefore, after reconstitution, the concentration of ixabepilone in the solution was 2 mg/mL. [The diluent supplied consists of a sterile nonpyrogenic 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 \(-80^\circ\text{C}\) until use. On each experimental day, the stock solution was diluted by adding 50% ethanol/50% cremophor with subsequent vortexing to yield a resultant solution that was 5 times the required dosing concentration. Finally, 4× volumes of PBS were added, while vortexing, to achieve a final dosing concentration of 10 mL/kg.

Animals

Female BALB/c mice (approximately 7–8 weeks of age at onset of dosing) were used for all experiments. Mice were obtained from the Harlan Laboratories Inc. and maintained with \textit{ad libitum} access to water and a standardized synthetic diet (Harlan Teklab), both before and during the course of the study.

Animal housing and procedure

Room temperature and humidity were maintained at 20 \pm 2°C and 55 \pm 10%, respectively. Artificial lighting provided a 12-hour light/12-hour dark cycle (light 7 AM–7 PM). All experimental protocols were approved by the Institutional Animal Care and Use Committee of Eisai, Baltimore, MD, and conformed to all of the applicable institutional and governmental guidelines for the humane treatment of laboratory animals.

MTD determination

For each experimental compound, an MTD on a (Q2D \times 3) \times 2 weeks schedule (every other day for 3 injections with a 2-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 at which no more than 10% deaths occurred and/or at which no mice displayed more than 20% individual weight loss and/or overt clinical signs of distress and/or inability to eat and drink, thus requiring euthanasia.

Doses chosen for the MTD studies were based on antitumor activity and/or neuropathy-inducing doses in mice as previously described (12, 17, 19, 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 administered into the caudal vein at a volume of 10 mL/kg.

Mice were weighed 3 times a week during the 2-week treatment period and for 2 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 6 doses of chemotherapeutic/vehicle had been administered. day 26 refers to the final experimental day (2 weeks after completion of dosing). Clinical signs and survival were monitored daily.

**Nerve conduction velocity (NCV) and amplitude measurements**

Baseline NCV was measured 1 week prior to initiation of dosing in 50 mice. Mice were subsequently randomized into 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, 0.75 × MTD, 0.5 × MTD, or 0.25 × MTD) on a (Q2D × 3) × 2 week schedule. Posttreatment nerve conduction measurements were made 24 hours 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°C and 41.0°C. Platinum subdermal needle electrodes (Grass Technologies) 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 NCVs were recorded orthodromically from 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-millisecond duration) isolated from ground and produced by the MP100 (BIOPAC Systems Inc.). Each nerve segment stimulation was repeated for 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 in spite of increase in voltage. Neuroelectric signals were impedance matched and differentially amplified with a gain of 20,000 and a frequency band of 20 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 milliseconds, 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; 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 hairline); the stimulating cathode was positioned 35 mm further distally. Digital NCV was recorded using stimulation at the base of the second toe and recording at the level of the lateral malleolus. The distance traveled was measured for each mouse and, in general, was between 9 and 14 mm. The response latency at supramaximal stimulation divided by the distance between the electrodes used for recording and stimulating provides 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 DRG 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 microscopic determinations. After embedding, samples were cut in 1-μm semi-thin sections with a microtome RM2265 (Leica Microsystems GmbH), and sections were stained with toluidine blue for light microscopic examination under a Nikon E200 light microscope (Nikon). Section codes were masked before examination such that 2 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 and 1.75 mg/kg eribulin mesylate on a (Q2D × 3) × 2-week schedule displayed significant body weight losses averaging between 14% and 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 doses 1.25 mg/kg or less were minimal (<10%), and mice in these groups displayed no overt clinical signs. Weight lost 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 2 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 with the control, and a similar trend was noted for 0.75 × MTD (1.31 mg/kg). A possible explanation for this paradoxical finding may be dehydration of the tail in these high-dose groups. Because 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, as 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 0.5 × MTD (0.875 mg/kg) to MTD (1.75 mg/kg) doses of eribulin mesylate. At these doses, eribulin mesylate...
induced mild to moderate pathologic 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 between 0.75 × MTD to MTD of eribulin mesylate doses, whereas dark inclusions were only very rarely observed at the MTD (Fig. 6B).

Figure 1. Body weight of mice. Body weight of mice expressed as a percentage of their starting weight following treatment with vehicle versus eribulin mesylate (A), paclitaxel (B), and ixabepilone (C) on a (Q2D × 3) for 2 weeks' schedule.
Paclitaxel

As shown in Figure 1B, all mice receiving more than 30 mg/kg paclitaxel on a (Q2D × 3) × 2 week schedule suffered appreciable weight loss (>20% of original starting weight). Furthermore, mice in these groups displayed significant hindlimb 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 completing 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 Figure 1B. The MTD of paclitaxel was determined to be 30 mg/kg when administered according to this regimen.

After 2 weeks of dosing with paclitaxel, clear dose-dependent decreases in caudal NCV and amplitude were evident (Fig. 3). The MTD of paclitaxel (30 mg/kg) caused significant reduction in caudal NCV compared with control (P < 0.01), whereas MTD and 0.75 × MTD (30 and 22.5 mg/kg, respectively) caused significant reduction in caudal amplitude compared with the control (P < 0.001). Paclitaxel doses of...
0.5 × MTD and lower had no effect on caudal NCV and amplitude. Furthermore, MTD and 0.75 × MTD of paclitaxel (30 and 22.5 mg/kg, respectively) 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 pathologic 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.

Figure 3. Paclitaxel effect on NCV and amplitude. Paclitaxel at its MTD reduced caudal NCV (A) and caudal amplitude (B). C, paclitaxel had no significant effect on digital nerve conduction velocity. D, paclitaxel at its MTD and 0.75 MTD reduced digital nerve amplitude. Figure depicts mean ± SEM.
in groups treated with 0.75 × MTD (22.5 mg/kg) or 0.5 × MTD (15 mg/kg) paclitaxel (Supplementary Fig. S1A and B). Furthermore, in DRG, dose-dependent adverse effects of paclitaxel were evident. Paclitaxel at 0.5 × MTD (15 mg/kg; Supplementary Fig. S2A) caused formation of dark inclusions in the cytoplasm. In addition, DRG from the 0.75 × MTD (22.5 mg/kg) group had clear vacuolations in the cytoplasm of neurons as well as in satellite cells (Supplementary Fig. 2SB). At the MTD of paclitaxel (30 mg/kg), a proportion of neurons in the DRG were degenerating and

Figure 4. Ixabepilone effect on NCV and amplitude. A, ixabepilone at 0.5 × MTD, 0.75 × MTD, and MTD decreased caudal NCV. B, ixabepilone at 0.75 × MTD and MTD produced dose-dependent decreases in caudal nerve amplitude. Ixabepilone at MTD caused a significant reduction in digital NCV (C) and amplitude (D). Figure depicts mean ± SEM.
their cytoplasm appeared much darker than normal neurons (Fig. 6C). Dose-dependent axonal degeneration was present in the proximal axons at 0.5 × MTD and greater (Fig. 6C; Supplementary Fig. 2S).

Ixabepilone

All mice receiving ixabepilone doses greater than 3.0 mg/kg on a (Q2D × 3) × 2-week schedule suffered appreciable weight loss (more than 20% of original weight) and displayed overt clinical signs of non–well-being (including piloerection and unkempt coats); these animals were euthanized before 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 dose of ixabepilone caused minimal weight loss (<10%), no overt clinical signs, and was well tolerated by mice. Minimal clinical signs were observed in the 2.75- and 3.0-mg/kg dose groups. The MTD of ixabepilone on a (Q2D × 3) × 2-week schedule was determined to be 3 mg/kg (Fig. 1C).

After 2 weeks of dosing with ixabepilone, a clear dose–response for decreasing caudal NCV and amplitude was evident (Fig. 4A and B). Doses of 0.5 × MTD to MTD (1.5–3 mg/kg) caused significant reductions in caudal NCV, whereas doses of 0.75 × MTD to MTD (2.25–3 mg/kg) caused significant reduction in caudal amplitude. At MTD (3.0 mg/kg), ixabepilone significantly reduced both digital NCV and amplitude (Fig. 4C and D).

Ixabepilone at doses below the MTD (0.75–2.25 mg/kg) generally had minimal effect on the morphology of nerve fibers, although rare and sporadic alterations were present in sciatic nerves (Supplementary Fig. S1C). Sciatic nerves from mice receiving MTD of ixabepilone (3 mg/kg) showed severe and frequent pathologic changes, represented by different stages of axonal degeneration. As shown in Figure 5D, axonal degeneration seemed to affect both small- and large-diameter fibers, although no regenerative figures (e.g., thin myelinated fibers) were present. In contrast, sciatic nerves from mice receiving MTD of ixabepilone (3 mg/kg) showed severe and frequent pathologic changes, represented by different stages of axonal degeneration. As shown in Figure 5D, axonal degeneration seemed to affect both small- and large-diameter fibers, although no regenerative figures (e.g., thin myelinated fibers) were present. Ixabepilone, at its MTD, caused very severe, frequent, morphologic alterations, both in neuronal and in glial compartments (Fig. 6D) of 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. Furthermore, adverse effects of ixabepilone administration were evident with other doses. DRG from mice receiving ixabepilone at 0.25 × MTD and

Figure 5. Effect of MTDs of eribulin mesylate, paclitaxel, and ixabepilone on sciatic nerve morphology. Morphologic changes were most severe with paclitaxel (C) and ixabepilone (B), with both agents causing frequent and severe pathologic 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 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 µm.
0.75 × MTD displayed occasional cytoplasmic swelling in the satellite cells (Supplementary Fig. S2C and D). Cytoplasmic dark inclusions and degenerating neurons, as well as vacuoles of satellite cells, were also evident in the 0.75 × MTD and MTD ixabepilone groups (Fig. 6D; Supplementary Fig. S2D). 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, and it has a significant negative impact on the quality of life and often results in treatment delays or discontinuation (25–27). Indeed, neurotoxic side effects associated with chemotherapies are second in frequency only to hematologic toxicities. Unlike the hematologic 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 analogues (e.g., cisplatin, oxaliplatin, carboplatin), taxanes (e.g., paclitaxel, docetaxel), Vinca alkaloids (vinristine), and, more recently, thalidomide and bortezomib as well as epothilones (9, 30). The degree and type of neuropathy depend on the chemotherapeutic 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). In spite of 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, because 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 neurophysiologic deficits and morphologic...
alterations in DRG and myelinated nerve fibers (23, 34–36). Somewhat surprisingly, in spite of 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 nontaxane 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 undergoing 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, compared with those of paclitaxel or ixabepilone, in mice.

All 3 chemotherapeutic drugs have antineoplastic activity in various animal models (6, 7, 17) and human cancer conditions (2, 37, 38). Importantly, all 3 compounds have shown efficacy in multiple in vivo cancer efficacy models at doses often less than the MTD described in this study in the case of eribulin mesylate (17), or generally comparable with the MTD, in the case of paclitaxel (17) and ixabepilone (6, 7).

For example, eribulin mesylate has shown efficacy in a human breast (MDA-MB-435) cancer xenograft model in athymic mice at doses between 0.25 and 1.0 mg/kg using a (Q2D × 3) × 4 weeks schedule (17). These efficacious doses are lower than the MTD described in this study (1.75 mg/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; ref. 17). Ixabepilone has shown antitumor activity in mouse models, including for breast cancer [KPL4, Pat-21], at a dose range of 3.2 to 6.0 mg/kg administered Q2D × 5 or Q4D × 3 (6, 7), which is also similar to the ixabepilone MTD (3 mg/kg) employed in this study. Therefore, it is reasonable to conclude that the mice used in this study received similar (paclitaxel, ixabepilone) or greater (eribulin mesylate) exposures than those required for efficacy.

Because these drugs have different potencies and pharmacokinetics, we compared them on the basis of MTD by using a neuropathy-inducing dosing regimen (Q2D × 3) for 2 weeks. Once MTDs were defined as 1.75 mg/kg eribulin mesylate, 30 mg/kg paclitaxel, and 3.0 mg/kg ixabepilone, we administered 0.25 × MTD, 0.5 × MTD, 0.75 × MTD, and MTD and evaluated their effects on NCV, amplitude, and morphology of sciatic nerves and DRG. Using these comparisons, ixabepilone and paclitaxel dosed on a (Q2D × 3) for 2 weeks schedule were found to produce significant deficits in conduction parameters of caudal and digital nerves at doses of 0.5 × MTD or more. 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 morphologic 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. Because all 3 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 because they promote polymerization of microtubules and increase polymer mass in cells. In contrast, eribulin mesylate is a microtubule-destabilizing agent because 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 has at least 10-fold lower affinity than at the positive 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 close to the same, binding site on β-tubulin (10, 43). As neuronal microtubules seem 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.

In spite of 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 clinical setting, eribulin mesylate could have a positive impact on the side effect profile of cancer patients receiving microtubule-based therapies.

Disclosure of Potential Conflicts of Interest

K.M. Wozniak, B.S. Slusher, R.G. Lapidus, B.A. Littlefield, and Y. Wu were employees of Eisai at the time this work was performed. K.M. Wozniak, Y. Wu and B.S. Slusher currently have a sponsored research agreement with Eisai. No other conflicts of interest were disclosed.

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