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Vincristine-induced allodynia in the rat

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Abstract

The aims of this study were two-fold: first, to simplify the method for creating a recently described neuropathic pain model in the rat, and second, to evaluate the effects of a number of drugs with analgesic or antihyperalgesic properties, in this model. Continuous intravenous vincristine infusion (1–100 μg kg^{-1} day^{-1}) for 14 days resulted in a dose dependent tactile allodynia (as measured by von Frey filaments) by 7 days at doses between 30 – 100μg kg^{-1} day^{-1}, with a hindlimb motor deficit observed at doses greater than 50 μg kg^{-1} day^{-1}. No thermal hyperalgesia was observed. Systemic morphine, lidocaine, mexiletine and pregabalin (given intraperitoneally) produced significant reduction of the allodynia, while tetrodotoxin was without effect. Continuous intravenous infusion of vincristine in rats thus provides a reliable model of chemotherapy induced neuropathy which may be used in defining the mechanism and pharmacology of this clinically relevant condition.

Keywords: Vinca alkaloids; Neuropathic pain; Allodynia; Chemotherapy; Hyperalgesia

1. Introduction

Cancer-related pain may result from direct or indirect effects of malignancies, from general effects of severe illness, and in addition from cancer therapies. Chemotherapy-induced peripheral neuropathy both results in patient suffering and limits further treatment with potentially useful anticancer drugs (Forman, 1990a,b). Vinca alkaloids (such as vincristine and vinblastine) have significant efficacy in the treatment of malignant tumors. These agents appear to exert their effects by binding to tubulin and interfering with microtubule dynamics, thus disrupting mitosis (Dumontet and Sikic, 1999). However, they also produce a variety of dose-limiting neuropathic conditions characterized as: (i) myalgias; (ii) painful burning paresthesias; (iii) glove-and-stocking sensory neuropathy and (iv) spontaneous hypersensitivity to otherwise mildly noxious (hyperalgesia) and frankly non-noxious (allodynia) stimuli. These effects in humans are dose and time dependent and often persistent. The hyperalgesia/allodynia may occur with latencies of days to weeks, and may be cumulative with similar toxicity due to other drugs used for the same malignancies such as paclitaxel (Ashburn and Lipman, 1993; Cavaletti et al., 1995; Forman, 1990a,b). Treatment of this painful condition has not been systematically addressed in the literature, although recommendations that apply to neuropathic pain in general are appropriate (Tyndel, 1994).

Animal models of painful neuropathies are useful in research aimed at both understanding the pathogenesis of these disorders, and evaluating potential treatments. We have modified the originally described rodent model of vincristine neuropathy (Aley et al., 1996) to produce a simpler method. The revised method obviates the need for daily intravenous injections for two weeks, by the use of a continuous infusion technique. We report our determination of the dose-response characteristics of this modified means of drug administration.

We have used the modified model to assess responses to drugs representing three major categories of agents used to treat chronic neuropathic pain: morphine, lidocaine, mexiletine, tetrodotoxin and pregabalin. The use of μ-opioid analgesics, including morphine, in neuropathic pain, has been a subject of controversy for some time; while limitations to their efficacy have been observed, they remain the general clinical criterion standard for potent analgesia (Portenoy et al., 1990). The well-described efficacy of

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systemic sodium channel blockers (antiarrhythmics, local anesthetics and anticonvulsants) such as lidocaine and mexiletine in neuropathic pain (Chabal et al., 1992; Kastrup et al., 1986; McQuay et al., 1995) has led to considerable interest in the possible role of dysfunctional voltage gated sodium channels as a pathological mechanism (Waxman et al., 1999). Pharmacological investigation of sodium channel subtype function is limited; while over 10 subtypes of sodium channel have been cloned, little is known of the relative affinities of available sodium channel blocking drugs for the various subtypes. The exceptions are the marine toxins, tetrodotoxin and saxitoxin, obviously not in clinical use, that block all known sodium channel subtypes with the exception of three that are relatively resistant to their effects. The tetrodotoxin-resistant subtypes include two forms exclusively expressed in sensory primary afferent fibers, and the cardiac-specific subtype. The usefulness of tetrodotoxin to distinguish whether these channels appear to have a significant role in neuropathic pain is recognized (Lyu et al., 2000). Lastly, gabapentin is used extensively in clinical practice to treat neuropathic pain; gabapentin and its analog, pregabalin, represent a distinct class of antihyperalgesics whose mechanism of action is unknown (Taylor et al., 1998), but which have defined efficacy in clinical and preclinical pain states, perhaps associated with inhibition of voltage-gated calcium channels.

2. Methods

2.1. Animals

Male Holtzman Sprague–Dawley rats (300–400 g; Harlan Industries, Indianapolis, IN, USA), housed in pairs under 12 h light/dark cycle, were used in the experiments. The experimental protocols were approved by the Institutional Animal Use and Care Committee of the University of California, San Diego, and respected the proposals of the Committee for Research and Ethical Issues of IASP. Precautions to minimize pathogen transmission to these potentially immunocompromised animals are already incorporated into standard protocols at our institution, and include the use of microfilter cage tops. Body weights were monitored on alternate days.

2.2. Vincristine administration

Vincristine (0, 1, 10, 30, 50 or 100 $\mu g\ kg^{-1}d^{-1}$) was intravenously infused continuously for 14 days using a mini-osmotic pump as follows. Vincristine (Sigma Chemical Co., St. Louis, MO, or Faulding, Cranford, NJ, MW = 923) was diluted with saline to the concentration calculated to produce the desired dose. The pumps (Alzet Model 2002, Alza Corporation, Newark, DE) were filled with the drug solution and primed by incubation at 37°C for 4 h before the surgery. Under halothane/oxygen anesthesia, catheters made from PE-60 tubing were inserted into an external jugular vein by cutdown. A small subcutaneous pocket was created in the posterior thoracic area into which the osmotic pump was placed. The catheter was tunneled subcutaneously and connected to the osmotic infusion pump, and all incisions were closed with 4.0 silk suture.

2.3. Tactile paw withdrawal threshold measurement

A series of von Frey filaments with exponentially incremental stiffness (0.4, 0.7, 1.2, 2.0, 3.6, 5.5, 8.5 and 15.1 g) (Stoelting, Wood Dale, IL, USA) was used as previously described to measure the 50% threshold for hindpaw withdrawal in awake, unrestrained rats. Briefly, rats were placed in a plastic cage with an open wire mesh bottom and allowed to acclimate for several minutes. Brisk paw withdrawal from the pressure of a filament gently bent against the plantar paw was defined as a positive response, and absence of withdrawal within 6 s as a negative response. Filaments were touched to the hindpaw in sequential ascending or descending order until the threshold of response was crossed. Each time the threshold was crossed, the direction of stimulus presentation was reversed and the procedure was resumed. Four responses were collected after the first threshold detection, and the 50% withdrawal thresholds were interpolated. In cases where response thresholds fell outside the range of detection, 15.00 and 0.25 g were respectively assigned for continuous negative or positive responses to the limit of stimuli. Responses of both hindpaws were averaged.

2.4. Thermal paw withdrawal latency measurement

Thermal nociceptive responses were assessed by a commercially available device (University Anesthesia Research Group, La Jolla, CA), which has been described previously. In brief, a calibrated lightbulb heat source was directed at the plantar surface of one hind paw of a rat placed on a heated (30°C) glass surface. The interval between the start of the heat stimulus and the paw withdrawal was measured and defined as paw withdrawal latency (PWL:s). PWL was measured for both hindpaws and the average was calculated.

3. Experimental Protocols

3.1. Vincristine infusion effects on tactile and thermal thresholds, and general health

Von Frey filament withdrawal thresholds were determined on day −1 and 0 (pre-pump implantation) and on days 3, 6, 8, 10, 12, and 14. Thermal paw withdrawal latency was determined on the same days only for the saline, vincristine 50 and 100 $\mu g\ kg^{-1}\ d^{-1}$ infusion groups. General health of the animals was checked daily and body weights were checked before each testing session. Ambulation was observed, and stepping and righting responses were tested to

assess motor function. Rats with >20% body weight loss or motor deficits were not used for drug testing. The vincristine infusion dose producing significant paw threshold reductions without major detriment to general health was selected for further studies. A group of five rats was kept for 3 weeks after the cessation of vincristine infusion to observe for spontaneous recovery from the neuropathy.

3.2. Effects of systemic drug treatment on vincristine induced allodynia

Separate groups of animals were prepared for the following experiments using the optimum vincristine infusion dose as determined above. Drugs were given intraperitoneally (IP) after the establishment of significant allodynia on infusion day 8. Drugs tested were: morphine sulfate (5 mg kg^{-1}: MW = 668.8; Merck & Co., West Point, PA), lidocaine hydrochloride (45 mg kg^{-1}: MW = 270.8, Abbott Laboratories, North Chicago, IL), mexiletine (30 mg kg^{-1}, MW = 215.73, Celgene, Warren, NJ), tetrodotoxin (8 μg kg^{-1}, Sigma Chemical Co, MW = 319.3), and *S*(+)3-isobutyl-gamma-aminobutyric acid (pregabalin) (80 mg kg^{-1}: MW = 157; Parke-Davis, Ann Arbor, MI). Rats were randomly assigned to receive the treatment drugs; however, no rat received the same treatment more than twice. Thresholds were measured 15, 30, 45, 60 and 90 min after the IP injection of the drug. Rats were used up to four times, with 48 h washout periods after each drug. At the beginning of each testing session, thresholds were verified to have completely returned to pre-treatment baselines.

3.3. Data analysis

Tactile paw withdrawal thresholds (g) were compared

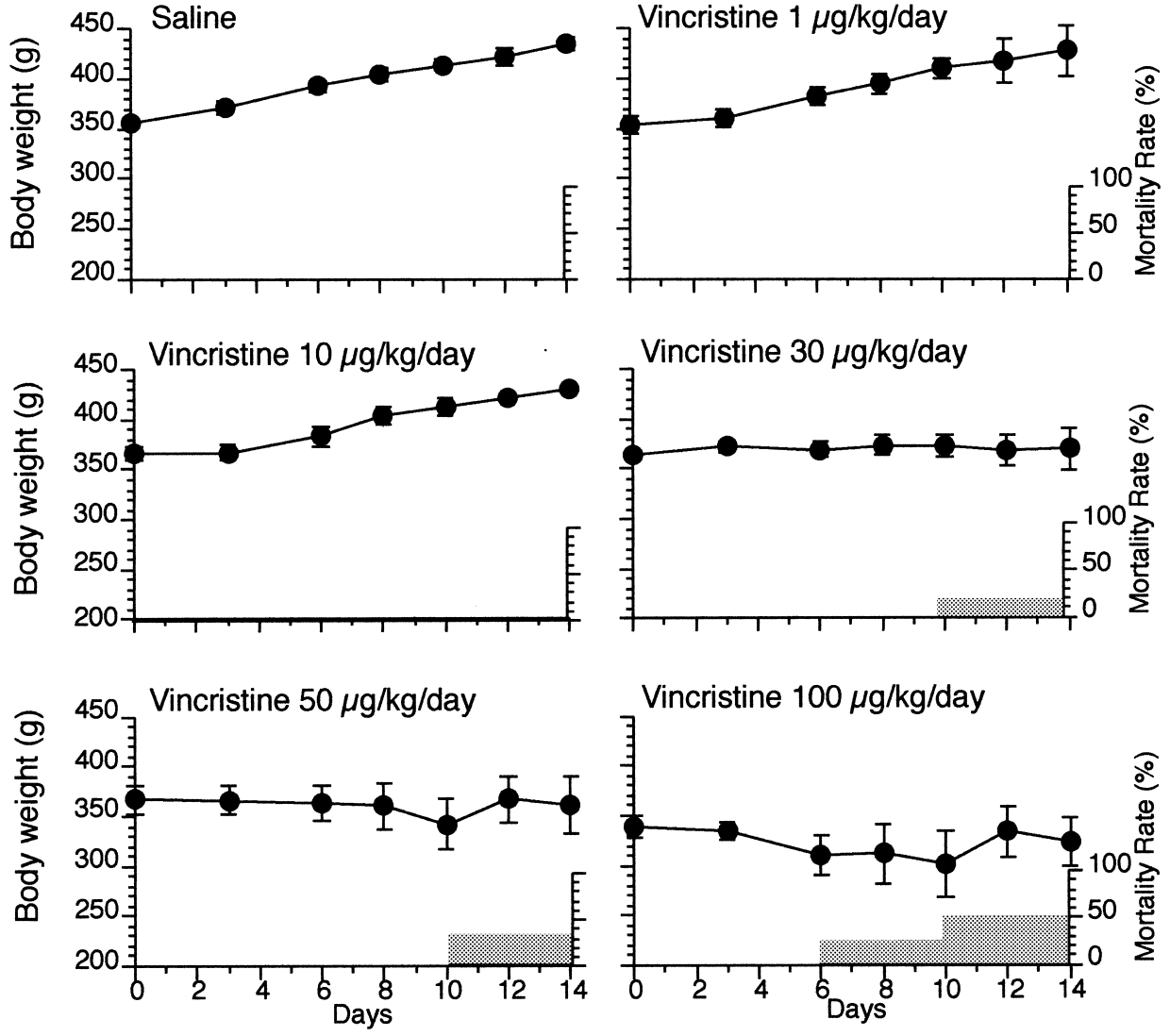

Fig. 1. The panels illustrate daily weights and survival curves for groups of rats (n = 4–6) receiving indicated infusion rates of vincristine, IV. The x-axis (time) indicates days post initiation of vincristine infusion IV. The left y-axis illustrates the mean daily weights (g) ±SEM. The right y-axis illustrates cumulative percent mortality in each group.

using non-parametric statistical methods, as the computed results do not yield a mathematical continuum. Results are presented as median values. For paired comparisons, Wilcoxon's signed rank test was used. For repeated measures, Friedman's test was used followed by multiple comparisons as described by Siegel and Castellan (modified Dunnett's test). An allodynia index was computed to compare the effects of increasing doses of vincristine at producing allodynia in previously normal rats, by normalizing results on a scale defining the pre-treatment threshold (near 15 g) as 0% effect, and a threshold value of 0.25 g as 100% effect, using the following formula

$$\%\ \text{allodynia} = \frac{[\text{baseline (g)} - \text{post drug threshold (g)}]}{\text{baseline (g)}} \infty 100$$

To compare dose and drug effects, raw thresholds were normalized as percent of maximum possible drug effect (%MPE) using the following formula

$$\%\ \text{MPE} = \frac{[\text{post drug threshold (g)} - \text{baseline (g)}]}{[15\ \text{g} - \text{baseline (g)}]} \infty 100$$

in which pre-treatment (baseline) thresholds were assumed to reflect 0% drug effect and thresholds attaining the maximum (cut-off) value of 15 g were designated as 100% effect. %MPE values were compared using analysis of variance (ANOVA) followed by Fisher's PLSD test. Area under the curve (AUC) was calculated using the trapezoidal rule. Paw withdrawal latencies to thermal stimulus were compared using two way ANOVA. Results are presented as mean ± SEM. A P-value of <0.05 was considered significant. Statistics were performed using Statview 4.5® (SAS Institute Inc., Cary, NC) for the Macintosh.

4. Results

4.1. General effects

Mean body weights and cumulative mortality for all dose groups are shown in Fig. 1. Morbidity consisted chiefly of weight loss and motor dysfunction. Continuous infusion of vincristine 100 μg kg^{-1} day^{-1} ($n = 4$) resulted in >20% weight loss, motor dysfunction and 50% mortality. The apparent slight increase in weight after day 10 is an artifact created by the dropout of the most severely affected animals. Continuous infusion of vincristine 50 μg kg^{-1} day^{-1} ($n = 6$) also caused weight loss and 33% mortality; no rats showed motor dysfunction. Rats in the 30 μg kg^{-1} day^{-1} group ($n = 6$) maintained their weight during the infusion; one rat died. Vincristine doses lower than 30 μg kg^{-1} day^{-1} (10 μg kg^{-1} day^{-1}, $n = 5$; 1 μg kg^{-1} day^{-1}; $n = 5$) did not cause weight loss or death. In addition, rats receiving continuous vincristine infusions developed respiratory problems characterized by apparent dyspnea with wheezing or stridor. This occurred occasionally at 30 μg kg^{-1} day^{-1} and reliably at the higher doses.

4.2. Allodynia

No difference was found between pre-infusion thresholds on day −1 and day 0 (15 g (11.442–15) and 15 g (10.981–15), respectively ($P > 0.8$, Wilcoxon signed rank test)); accordingly, thresholds measured on day 0 were used as control thresholds. The vincristine infusion caused marked

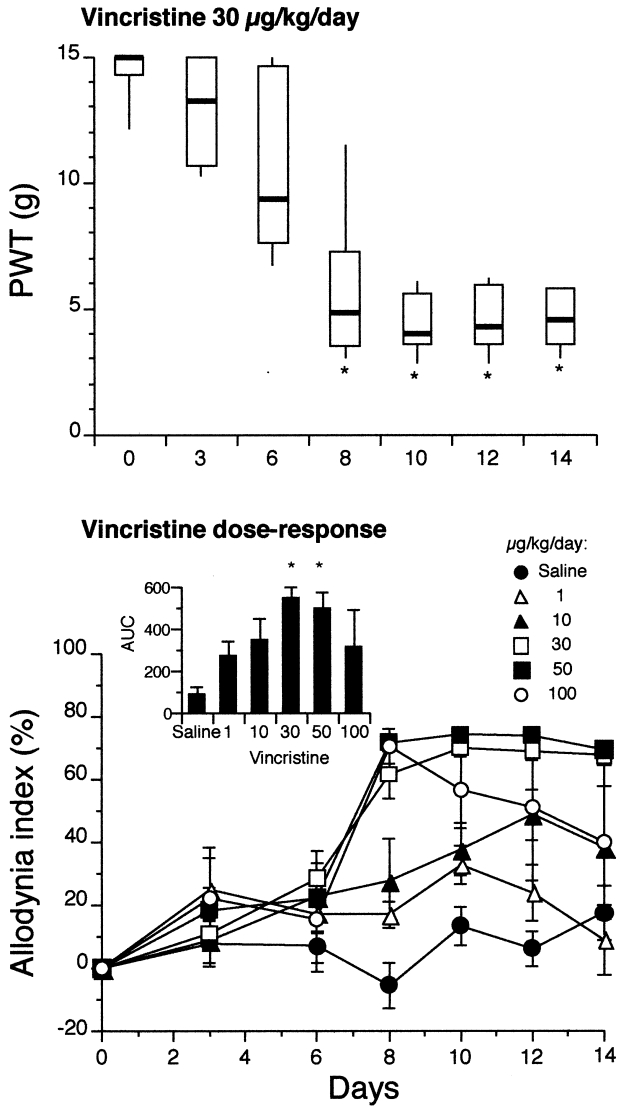

Fig. 2. The top panel represents the 50% paw withdrawal thresholds (PWT, g) (y-axis) of a group of 10 rats receiving continuous infusion of vincristine IV, 30 μg kg^{-1} d^{-1}. The x-axis of the box and whisker plot shows the day of vincristine infusion. Heavy bars denote the median values, boxes denote interquartile distances, and whiskers denote range of data. Values for days 8 through 14, inclusive, are significantly different from day 0 by Friedman's test followed by multiple comparisons ($P < 0.0001$). The bottom panel illustrates the dose-response curve for six doses of continuous vincristine infusion: (μ kg^{-1} d^{-1}) 0, 1, 10, 30, 50 and 100. Each group consisted of 4–6 rats. x-axis: days after initiation of vincristine infusion. y-axis: allodynia index, where 0 = normal, pre-drug threshold and 100% = maximally altered (allodynic) threshold (see Section 2 for computation). Error bars = SEM. Bar graph inset depicts the mean area under the curve (AUC) for each group as a dimensionless number; error bars = SEM (see Methods).

allodynia beginning on day 8 (Fig. 2). The time course of development of allodynia for the 30 μg kg^{-1} day $^{-1}$ infusion is presented as raw threshold data in Fig. 2, showing a significant time dependent reduction of the mechanical threshold (Friedman's test: $P < 0.0001$). The dose-response curve for allodynia was biphasic, with a peak at 30 μg kg^{-1} day $^{-1}$ (Fig. 2B).

In contrast with the severe tactile allodynia, no significant changes in thermal withdrawal thresholds were observed at either 50 μg kg^{-1} day $^{-1}$ (Fig. 3) or 100 μg kg^{-1} day^{-1} (data not shown).

4.3. Analgesic drug effects

Based on the preceding studies, the vincristine dose of 30 μg kg^{-1} day $^{-1}$ was chosen for further testing. This dose produced maximum allodynia while preserving general function. A separate group of animals ($n = 10$) was infused with vincristine 30 μg kg^{-1} day $^{-1}$ and tactile thresholds were tested. Animals with baseline thresholds below 6 g for both paws and below 4 g for at least one paw were used to test the systemic effects of drugs.

Intraperitoneal (IP) injection of morphine, 5 mg kg^{-1}, effectively reversed the allodynia. Lidocaine, 45 mg kg^{-1} IP, also significantly reversed allodynia. Pregabalin, 80 mg kg^{-1} IP, was observed to have a slower onset but a longer duration of anti-allodynic effect. Mexiletine, 30 mg kg^{-1} IP, had a marked effect with a prolonged duration of action (over 2 h). Preliminary dose ranging studies demonstrated a very narrow dosing window for tetrodotoxin, with limiting effects of unacceptable weakness leading in some cases to death, likely due to respiratory compromise. The highest usable dose, 8 μg kg^{-1} IP, had no effect on allodynia and indeed appeared to be without overall observable effects of any kind (Fig. 4).

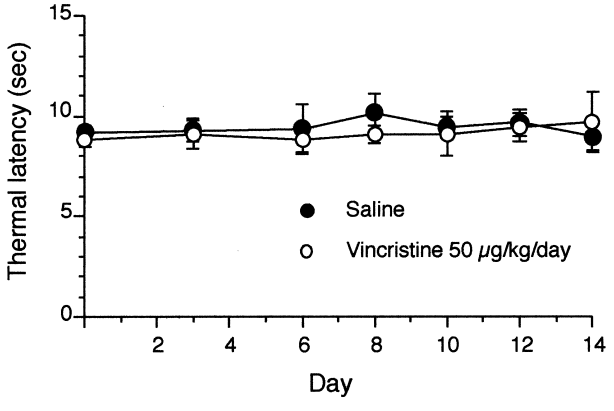

Fig. 3. Mean thermal paw withdrawal latencies for rats having received continuous infusions of vincristine, 50 μ k^{-1} d^{-1}, or saline ($n = 6$ each group). *x*-axis: day of vincristine infusion; *y*-axis: seconds elapsed prior to paw withdrawal from thermal stimulus. No statistically significant difference exists between groups.

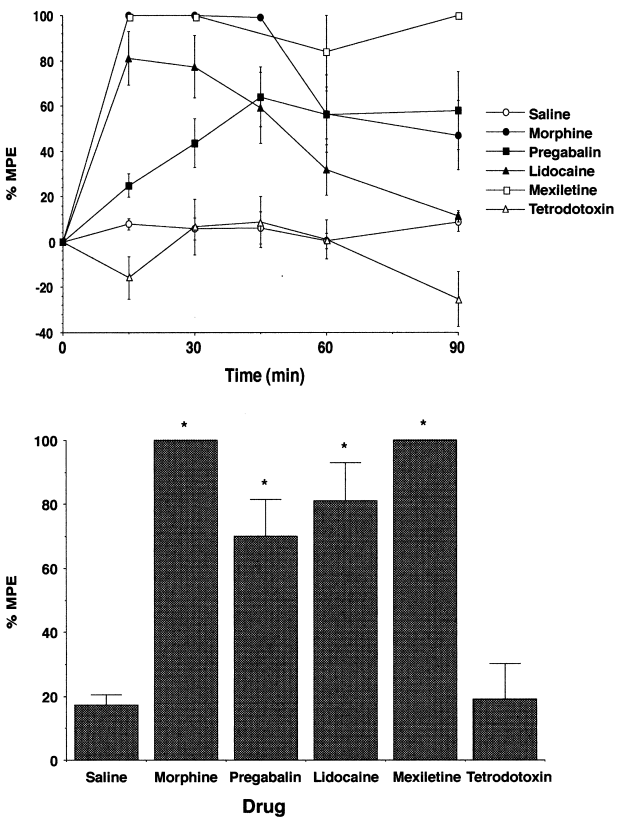

Fig. 4. The top panel illustrates time course data after administration of analgesic/anti-hyperalgesic drugs. *x*-axis: minutes after IP drug injection; *y*-axis: percent of maximum possible drug effect (%MPE). Drugs were tested at the maximum tolerated dose that did not produce limiting behavioral effects: morphine (5 mg kg^{-1}), pregabalin (80 mg kg^{-1}), lidocaine (45 mg kg^{-1}), mexiletine (30 mg kg^{-1}) and tetrodotoxin (8 μg kg^{-1}). $n = 5$–6 per group. The bottom panel compares the mean maximum effects of each drug; all drugs except tetrodotoxin were significantly different from saline, $P < 0.0001$, one-way ANOVA, Fisher's PLSD.

4.4. Recovery from allodynia

The five healthiest animals from the drug treatment studies group were kept for further testing to examine the reversibility of the neuropathy. In these animals, no significant recovery of the allodynia was observed by day 34 (3 weeks after the completion of the 2 week drug infusion), though all animals showed signs of slow body weight recovery (Fig. 5).

5. Discussion

5.1. Intravenous infusion model

The present study confirms and extends previous findings that prolonged intravenous administration of vincristine evokes dose-dependent tactile allodynia (Aley et al., 1996; Authier et al., 1999). Our method differs from previous reports in that we used a continuous infusion technique,

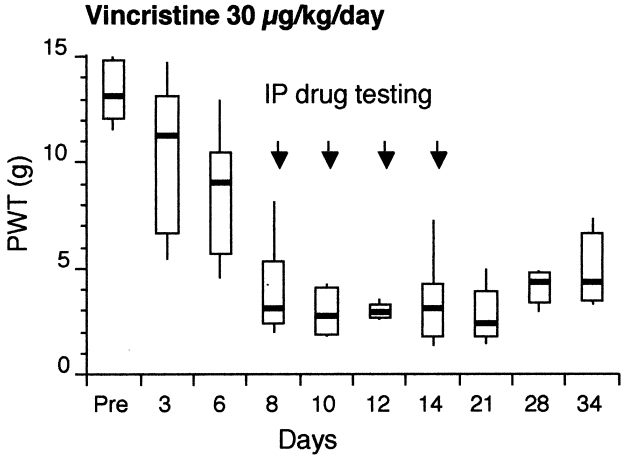

Fig. 5. Box and whiskers plot of paw withdrawal thresholds for rats receiving 30 μg kg^{-1} d^{-1} continuous vincristine infusion. Testing of analgesic drugs took place on indicated days. These rats were followed for 34 days post infusion (20 days after cessation of infusion) with minimal return of threshold toward baseline. Heavy bars = median PWT values. $n = 10$ (days 0–14), 5 (days 15–34).

which is considered to provide a consistent blood concentration of the drug.

This method provided a convenient alternative to repetitive daily intravenous injections over a 14 day period. The disadvantages of daily injections include: (1) the need to firmly restrain or anesthetize the animals daily, in order to perform a painful injection with precision; (2) considerable labor of 14 consecutive daily drug dose preparations and administrations by tail vein injection; (3) significant risk of drug extravasation with resulting tissue scarring or ulceration from this irritating agent, as well as improper delivery; (4) progressive decrease in ease of venous access with repetitive venipuncture; (5) risk to laboratory personnel of needlestick with a chemotoxic drug. Implantation of the pumps, in contrast, is a simple procedure that need only be performed once, provides reliable intravenous drug delivery, takes little time to perform, and is well tolerated.

It required less drug using the implanted pumps (30 μg kg^{-1} day^{-1}) to achieve the same endpoint, of demonstrable neuropathy without unacceptable additional morbidity, as intermittent IV bolus dosing (50–100 μg kg^{-1} day^{-1}). These results appear to indicate that intermittent dosing, as is clinically practiced in chemotherapeutic administration of vincristine, allows for some recovery from toxic effects in between boluses; indeed, standard prescribing information recommends dosing no more frequently than every 7 days to minimize toxic manifestations (Calabresi and Chabner, 1990). Since our goal was to recreate the toxic side effects that remain dose-limiting in humans, we found this paradigm to be useful and efficient.

The absence of thermal hyperalgesia in this model showing robust allodynia is of great interest, and was also specifically noted in previous studies of this model (Aley et al., 1996; Authier et al., 1999). While one electrophysiological study, interested primarily in C-fibers, has demonstrated C-fiber nociceptor hyper-responsiveness in this model in a teased sural nerve fiber preparation, these responses were noted to be only to suprathreshold stimulation, and only occurred in additionally mechanically responsive fibers (Tanner et al., 1998b). A recent histological study from the same group noted most changes in large diameter sensory neurons in the dorsal root ganglia neurons (Topp et al., 2000), suggesting that large myelinated sensory fibers, transducing sensations of light touch and vibration, may bear the brunt of the toxicity. Neuropathic pain is not invariably associated with thermal hyperalgesia; in a clinical study of patients with peripheral nerve injuries, all of whom had spontaneous pain and allodynia to vibration, decreased thermal pain thresholds were associated with milder, compression type nerve injuries, whereas in patients with more severe nerve injuries, thermal pain thresholds were normal (Wahren et al., 1991). Of note, patients in the former group were more likely to benefit from intravenous regional guanethidine therapy, suggesting that somewhat different mechanisms may have been dominant. The separability of allodynia and thermal hyperalgesia in this neuropathic pain model further supports the likelihood that these two manifestations of the neuropathic state have differing pathophysiological etiologies.

Side effect profiles were significant with the continuous infusion method. Body weight loss was more prominent than that reported with intermittent injections. In addition, a respiratory problem characterized mainly by wheezing became apparent in some rats receiving doses of 30 μg kg^{-1} day^{-1} or higher. We did not determine the basis of this problem. An infectious etiology resulting from vincristine-related immune compromise in the face of an ordinarily subclinical pathogen, latent in the colony from whence these animals originated, is very likely. Less likely causes include allergic or asthmatic responses to the drug as have been described (Thomas et al., 1993). Additionally, cranial nerve involvement resulting in bilateral vocal cord paresis, leading to stridor, has been reported with vincristine infusion in man (Burns and Shotton, 1998).

In humans, vincristine-induced neuropathies may persist for extended intervals after the termination of chemotherapy (Postma et al., 1993). Aley et al. (1996) showed recovery of the neuropathy in 2 weeks in their model. In the present continuous infusion model, recovery of allodynia induced by the vincristine infusion was not observed up to 3 weeks after cessation of drug infusion. Thus, the present model appears to be somewhat more robust, and to more accurately represent the clinically observed persistence of vincristine induced neuropathy.

5.2. Vincristine neuropathy

The mechanisms whereby the vinca alkaloids cause neuropathic pain states are poorly understood. The classical clinical features of symmetrical peripheral neuropathy are

present; the earliest and most consistent sign of this toxicity is noted to be depression of deep tendon reflexes, and further features include paresthesias, dysesthesias, and numbness of the distal extremities. Autonomic neuropathy may develop, with severe constipation, as well as muscular weakness. The typically poor ability of these parenterally administered drugs to penetrate the blood brain barrier, along with signs of peripheral neurologic dysfunction as above, strongly suggest that the sensory effects are due to actions on primary afferent neurons. While neurotoxicity is considered a frequent and dose-related complication of vincristine therapy (Calabresi and Chabner, 1990), there have been few systematic studies of this phenomenon in the clinical literature; more recently, the availability of a rodent model has provided material for preclinical studies. Decreased microtubular density can be visualized in unmyelinated axons after vincristine administration (Tanner et al., 1998a). The toxicity may be more pronounced at the level of the dorsal root ganglion cell, which lies outside the blood brain barrier. Effects upon dorsal root ganglia that may precede changes in the peripheral axon have been demonstrated in a recent study, demonstrating swelling of large diameter dorsal root ganglion neurons with neurofilament accumulation. Disruptions of axonal transport associated with the changes in microtubular dynamics are a likely factor in the creation of these neuropathic effects (Topp et al., 2000). One potential mechanism could involve nuclear deprivation of retrograde trophic influences derived from target organ innervation (skin, muscle) with consequent effects on gene regulation.

While the present studies do not shed light upon the precise mechanisms whereby the action of vincristine leads to allodynia, preclinical investigations of allodynia in other forms of peripheral nerve injury have shown the importance of the association with upregulation of sodium channels (Boucher et al., 2000) and the activation of spinal glutamate receptors (Calcutt and Chaplan, 1997; Chaplan et al., 1997). Further studies using the present model of vincristine infusion may be useful in defining changes that are common to other painful neuropathies.

5.3. Action of analgesic agents

Importantly, these preclinical nerve injury models have helped in the development of analgesic strategies useful in clinical settings. Several classes of agents have been demonstrated to be effective. Tactile allodynia has been shown to be particularly responsive to the delivery of systemic sodium channel blockers at low concentrations that do not produce measurable axon blockade, reflecting the potential role of upregulated sodium channels. Both lidocaine and mexiletine, sodium channel blockers, given systemically, are used clinically to alleviate neuropathic pain (Dejgård et al., 1988; Kastrup et al., 1986). These agents were effective in reducing the allodynia induced by vincristine infusion. Use of sodium channel blocking drugs has not been specifically reported in vincristine neuropathy; purely anecdotal support for the efficacy of mexiletine in this setting, from the authors' clinical experience does however exist (Chaplan, unpublished).

Of note, the sodium channel blocker systemic tetrodotoxin showed no effect on allodynia within doses tolerated by the awake rat. Tetrodotoxin has of course no therapeutic applications; its usefulness lies in its degree of sodium channel subtype selectivity. The category of tetrodotoxin-resistant or insensitive channels is presently known to consist of three members: SNS/PN3 (SCN10A) and NaN/SNS2 (SCN11A), both sensory neuron-specific, and the cardiac-specific sodium channel, rh1 (SCN 5A) (Akopian et al., 1996; Dib-Hajj et al., 1998; Rogart et al., 1989; Sangameswaran et al., 1996; Tate et al., 1998). Insofar as sodium channels may be important in this model, they appear to be less sensitive to tetrodotoxin than other sodium channels involved in more vital functions, precluding definitive assessment of their role in allodynia by this method. Since we do not know the actual concentration of tetrodotoxin at the effect site, it is not possible to conclude from these data that tetrodotoxin-resistant channels are responsible. However, these data do suggest that these channels may warrant further investigation.

Pregabalin, an analog of the anticonvulsant gabapentin, was also effective. Gabapentin has been shown clinically to have efficacy in treating a variety of neuropathic pain states. The use of this agent in treating the sequelae of vincristine or other chemotherapy-related neuropathic pain syndromes has not been reported. The mechanism of action of the gabapentinoids has not been established, though an effect mediated by an interaction with the $\alpha_2\delta$ voltage-dependent calcium channel subunit has been hypothesized (Luo et al. 2001).

Systemic morphine was also effective in reducing the allodynia. Substantial debate has centered on the role of opioids in the treatment of neuropathic pain. We have previously shown that activation of spinal μ opioid receptors does not appear to suppress allodynia in a different model, tight ligation of L5/6 spinal nerves, but that systemic morphine is effective, suggesting a role for brain, but not spinal, μ opioid receptors in this model (Lee et al., 1995). In contrast, spinal morphine significantly suppresses the allodynia associated with streptozotocin-induced diabetic neuropathy in the rat, suggesting that the role of μ opioid receptors differs in conditions of varying etiologies that give rise to allodynia (Calcutt and Chaplan, 1997). Additional work will be required to address whether spinal delivery of morphine is as effective against allodynia in this model as systemic delivery. In addition, since some of the controversy surrounding use of opiates in neuropathic pain centers on the accelerated development of tolerance, it will be of interest to experimentally address the considerations of opiate tolerance in vincristine neuropathy.

In summary, we have utilized a continuous infusion technique and extended the rodent model of vincristine induced

neuropathy. This model results in a reliable peripheral neuropathy that should prove useful in defining the mechanisms underlying allodynia, and devising/assessing potential treatments. The present work provides preclinical evidence in support of the potential utility of two relatively novel classes of agents in treating the associated pain component of the therapy induced neuropathy, systemic sodium channel blockers and the gabapentinoids. Both classes of agents are already employed in the treatment of other classes of neuropathic pain and the present studies suggest that they may have some therapeutic advantage in the palliation of vinca alkaloid induced allodynia.

Acknowledgements

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