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Effect of chronic vincristine treatment on mechanical withdrawal response and pre-pulse inhibition in the rat

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Abstract

Chemotherapeutic agents are associated with a number of serious side-effects. In addition to the development of peripheral neuropathy, patients often complain of additional symptoms related to attentional mechanisms. Although a great deal of interest is directed towards understanding the mechanisms underlying the development of peripheral neuropathy, there is a paucity of research that has examined the extent of impairment of attention in animals receiving chemotherapeutic agents. Therefore, the purpose of this experiment was to examine attentional mechanisms using the method of pre-pulse inhibition in animals that were chronically treated with vincristine. Although vincristine treated animals developed signs of peripheral neuropathy, there was no associated alteration of pre-pulse inhibition relative to vehicle treated animals. These results highlight the importance of continuing to develop methodology to model symptom burden in patients receiving chemotherapy.

Keywords: Chemotherapy; Chemotherapeutic treatment; Hyperalgesia; Allodynia; Attention

There are a number of effective and commonly used anti-neoplastic drugs for the treatment of a variety of tumors [6]. Unfortunately, virtually all of these compounds are associated with a number of serious side-effects. Perhaps one of the most common clinical issues related to chemotherapeutic neurotoxicity is peripheral neuropathy identified as numbness, tingling and burning pain of the hands and feet. Over the last few years, a great deal of research has focused on understanding the underlying mechanisms associated with the development of peripheral neuropathy with a number of commonly used chemotherapeutic agents [1,2,4,9,11].

In addition to the development of peripheral neuropathy, clinical signs of impairment of general cognitive impairment are also present in patients undergoing chemotherapy. The symptoms of such impairment include interruption of normal attentional and memory mechanisms [3,8]. Although the additional symptom burden has a significant impact on the quality of life, there remains a paucity of literature that has explored the underlying mechanisms associated with signs of cognitive impairment [10,12] and we are aware of no reports that have examined possible impairment of attentional mechanisms. Therefore, the purpose of this experiment was to explore the potential impairment of attentional mechanisms during treatment with vincrisitine. The experimental procedure utilized the well-developed method of pre-pulse inhibition to measure sensorimotor gating during the time of development of peripheral neuropathy.

Male Sprague–Dawley rats weighing 250–400 g at the beginning of the experiment were used. Animals were housed in pairs with free access to food and water. Vincristine is partially excreted in urine and feces, so the bedding was frequently changed and disposed of as biohazard waste.

Animals were randomly assigned to receive vincristine treatment or serve as vehicle injection controls. Vincristine hydrochloride (Sigma Co., St. Louis) was dissolved in physiological saline (0.1 mg/ml) and administered i.p. at a dose of 0.1 mg/100 g b.w. Vehicle treated animals were administered an equivalent volume of physiological saline. Animals were treated once per day during the 18-day period of drug treatment. Additional behavioral testing was performed after a 3-day washout period (day 22). To minimize the potential

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acute effects of vincristine, behavioral testing was performed approximately 21 h post-injection (12 p.m. injections, 9 a.m. behavioral testing) on days 5, 9, 14, 19, and 20.

Two behavioral test paradigms were utilized in the present experiment. First, mechanical paw withdrawal threshold testing was performed using the application of von Frey monofilaments to the plantar surface of the hindpaw. Animals were placed within a Plexiglas chamber (20 cm × 10.5 cm × 40.5 cm) and allowed a 15-min habituation period. The chamber was placed on top of a mesh screen to allow for easy administration of mechanical stimuli to the plantar surface of both hindpaws. Mechanical threshold measurements for each hindpaw were obtained using eight von Frey monofilaments (5, 7, 13, 27, 43, 64, 106, and 202 mN) using the up/down method [5]. Each trial started with a von Frey force of 13 mN delivered for approximately 1 s to the right and then the left hindpaw. For each paw, if a withdrawal response to the mechanical stimulus was not observed, then the next higher force was delivered. If a withdrawal response was observed, the next lower force was delivered. This procedure was performed until no response was observed at the highest force (202 mN) or until four stimuli were administered following the initial response. The 50% mechanical paw withdrawal threshold for each paw was calculated using the following formula: $[\mathrm{Xth}]\log = [\mathrm{vFr}]\log + ky$, where [vFr] is the force of the last von Frey used, $k = 0.2268$ which is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses [5]. If an animal did not respond to the highest monofilament (202 mN), then $y = 1.00$ and the 50% withdrawal response for that paw was calculated to be 340.5 mN. Threshold testing at each test period was performed three times and the scores averaged to determine the mechanical paw withdrawal threshold for each animal.

For measures of pre-pulse inhibition, animals were individually placed into 10 cm Plexiglas cylinders in a ventilated sound-attenuated test chamber with 65 dB background noise. The cylinders were placed onto an accelerometer to detect the motion of the rat within the cylinder. Five minutes later, startle amplitude was recorded in a session that included multiple trial types: (1) a 118-dB, 40-ms noise burst presented alone (“Pulse”); or (2) the same pulse preceded 60 ms by a 20-ms noise burst (“Pre-pulse”) that was 4, 8, or 16 dB above background (i.e. 69, 73, or 81 dB); or (3) no stimulus (“Nostim”). Pre-pulse stimuli in this range of intensities do not consistently elicit startle responses. These five trials were presented in a pseudorandom order with a variable inter-trial interval (average 15 s). A session consisted of 25 trials. For each pre-pulse intensity, the data was converted to a ratio of startle response relative to the 118-dB pulse using the formula ((response to 118 dB − response to pre-pulse)/response to 118 dB) and averaged across the test session.

As seen in Fig. 1, chronic administration of vincristine decreased paw withdrawal threshold. Statisitical analysis of

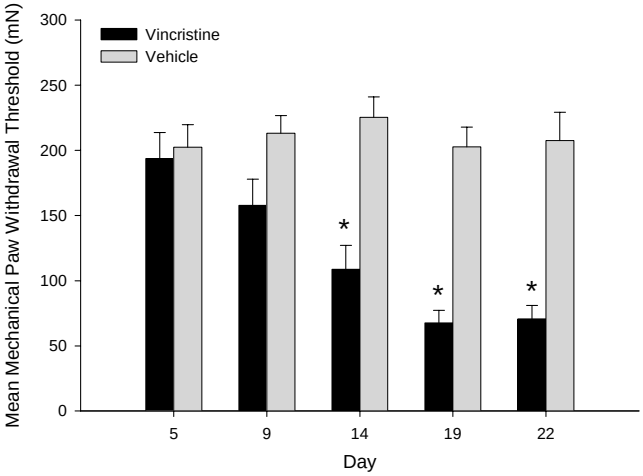

Fig. 1. Mean (±S.E.M.) mechanical paw withdrawal threshold across the test period for animals treated with vincristine ($n = 14$) or vehicle ($n = 12$). Drug treatment started on day 0, with animals receiving daily i.p. injection at 12 noon. Behavioral testing was performed the following day, prior to the test of pre-pulse inhibition and the next daily injection. Therefore, day 19 data indicates animals were treated for 18 days. Day 22 data was collected after a 3-day “washout” period. The onset of mechanical hypersensitivity was gradual with a statistically significant decrease in mechanical paw withdrawal threshold beginning at day 14. $^{*}P < 0.001$ vs. saline injected control.

mechanical paw withdrawal threshold revealed a significant group × test period interaction ($F(4, 92) = 7.21$, $P < 0.001$). Additional analysis (Tukey post hoc test) revealed that the onset of mechanical allodynia was gradual, with first signs of neuropathy evident at 9 days of injection. The magnitude of neuropathy was greatest at day 19 of testing and remained severe after 3 days of washout. Saline treated animals displayed constant mechanical paw withdrawal thresholds during the duration of the test period.

The effect of vincristine treatment on pre-pulse inhibition across the test days during the 18 days of drug treatment and 3-day washout period is illustrated in Fig. 2. As can be seen, vincristine treatment did not alter pre-pulse inhibition relative to vehicle treated controls ($P > 0.05$). Of primary importance is the lack of relationship between the development of the peripheral neuropathy and pre-pulse inhibition. At days 9–22, when vincristine treated animals were displaying significant mechanical allodynia, pre-pulse inhibition was not altered compared to vehicle treated control animals.

The effect of vincristine treatment on mechanical sensitivity supports a previous report of enhanced response to mechanical stimuli following a period of systemic vincristine administration [11]. The exact mechanisms underlying the enhanced mechanical response remain unknown, but appear to be largely mediated by altered activity in the dorsal horn [11]. Specifically, wide dynamic range neurons have enhanced evoked responses and display significant neuronal afterdischarge at a time when animals also display behavioral signs of mechanical hyperalgesia. It remains to be determined if similar underlying neural mechanisms are

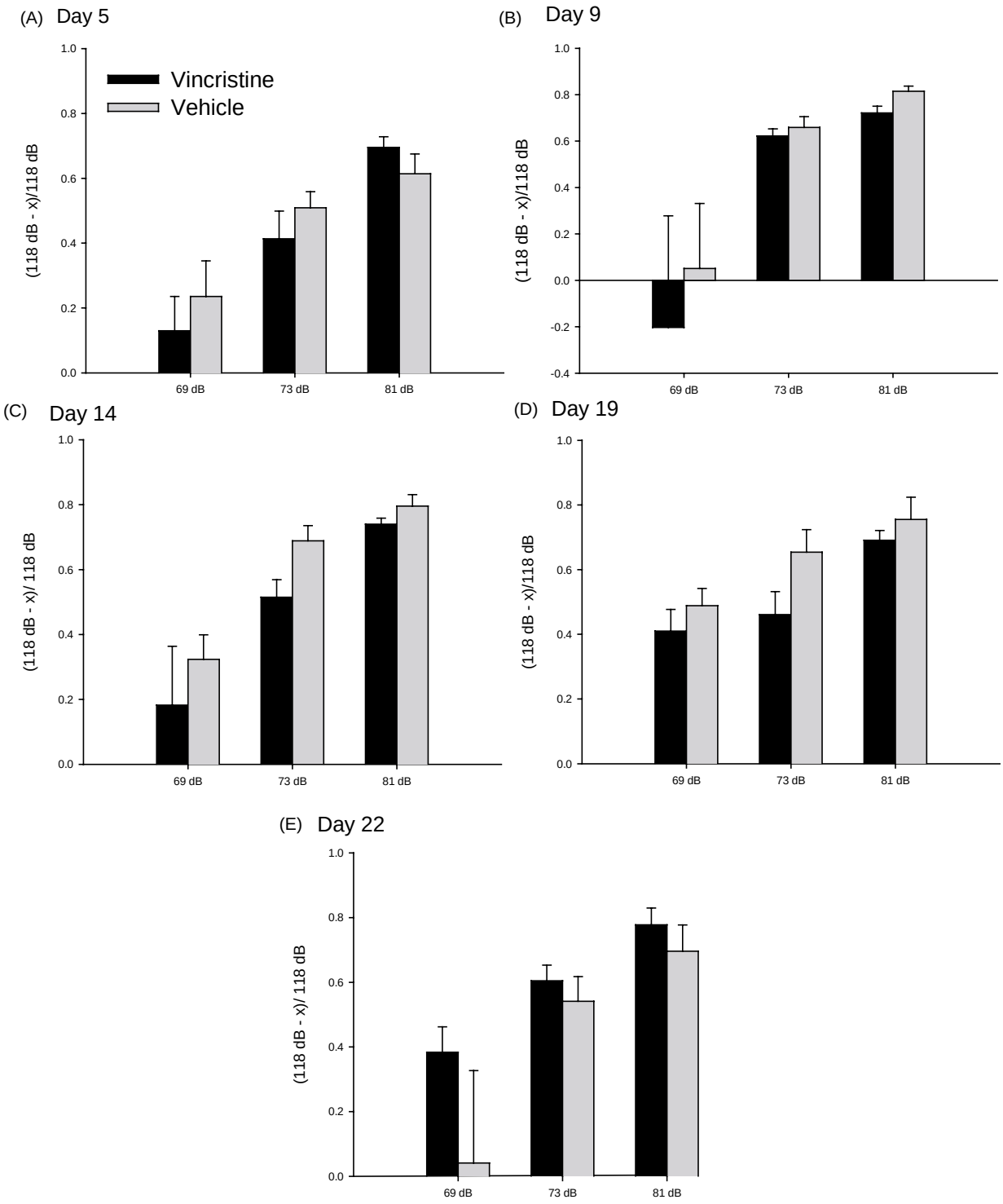

Fig. 2. Normalized startle response to different dB levels of tone across the test period for animals treated with vincristine ($n = 14$) or vehicle ($n = 12$). Drug treatment started on day 0, with animals receiving a daily i.p. injection at 12 noon. Behavioral testing was performed the following day after the test of mechanical sensitivity and prior to the next daily injection. Therefore, day 19 data indicates animals were treated for 18 days. Day 22 data was collected after a 3-day "washout" period. Vincristine treatment failed to alter pre-pulse inhibition at any time during the test period even though animals were displaying mechanical hypersensitivity beginning at day 14.

associated with the development of mechanical hyperalgesia following the administration of other chemotherapeutic agents.

Clinically, chemotherapy interrupts normal attentional and memory mechanisms [3,8]. In animals, disturbances of general cognitive function associated with chemotherapeutic agents remains unclear. For instance, methotrexate has been reported to impair [12] or have no effect [10] on measures of neuropsychological function (i.e. appetitive Pavlovian conditioning, conditioned taste aversion, etc.). Therefore, the main purpose of the present experiment was to test the hypothesis that chronic vincristine treatment would cause an impairment of attentional mechanisms.

Pre-pulse inhibition is a measurement of sensorimotor gating and is used to assess the underlying processes that reflect attention. In this paradigm, the response to an auditory stimulus is blunted by pre-exposure to a priming stimulus presented a brief (i.e. 50–300 ms) time earlier. The blunting in response is thought to result from an inhibitory control that modulates the processing of intense sensory input [7]. Attenuation of the inhibitory response is thought to reflect impairments in attentional mechanisms. The present data failed to find support for the hypothesis that vincristine treatment impairs attentional mechanisms. The lack of effect is unlikely due to the drug treatment paradigm since pre-pulse inhibition testing was performed on all animals during the time when the signs of peripheral neuropathy were present. The lack of positive findings with chronic administration of vincristine does not rule out the possibility that other aspects of attention and/or cognitive function are impaired or that other chemotherapeutic agents could have a significant effect on attentional mechanisms as measured using the pre-pulse inhibition paradigm. What is important, however, is that a dose regimen that induces signs of peripheral neuropathy is not associated with impairments in attentional mechanisms. Future experiments will continue to focus on measuring impairment of attentional and cognitive function during chemotherapeutic treatment with the ultimate goal of understanding and preventing symptom burden in cancer patients.

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