

Systemic Tizanidine Hydrochloride (Zanaflex™) Relieves Thermal Hyperalgesia in Rats with an Experimental Mononeuropathy

Allen H. Hord, MD, Amale G. Chalfoun, MD, Donald D. Denson, PhD, and M. Isabel Azevedo, MD

Division of Pain Medicine, Department of Anesthesiology, Emory University School of Medicine, Atlanta, Georgia

We sought to determine whether tizanidine, an α_2 -agonist, relieved thermal hyperalgesia in rats with surgically induced neuropathic pain. We used a Sprague-Dawley rat model in which a chronic constriction of the sciatic nerve caused the rats to develop postural changes, mechanical allodynia, and thermal hyperalgesia. Thermal hyperalgesia was verified through paw withdrawal latency (PWL). PWL was tested before surgery, after surgery, and after injections with tizanidine (0.5, 1.0, or 2.0 mg/kg) or normal saline. Ambulatory and total movements were evaluated by placing the rats

in activity cages. Thermal hyperalgesia was induced in all rats after surgery. Tizanidine, but not saline, caused a significant improvement in PWL ($P < 0.05$), with complete reversal of thermal hyperalgesia at all doses on postoperative Day 6. Rats who received tizanidine 2 mg/kg maintained complete reversal of thermal hyperalgesia through postoperative Day 9. Some sedation was observed with tizanidine 2 mg/kg, but not with smaller doses. We conclude that tizanidine effectively reversed thermal hyperalgesia in a rat model.

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α -Adrenergic agonists and antagonists are often used in the treatment of chronic pain in humans. The α -adrenergic antagonist, phentolamine, is often used as a test for sympathetically maintained pain (1), and oral terazosin has been recommended as a treatment for patients who respond to phentolamine (2). Clonidine, the α_2 agonist that has classically been used for pain control, has many side effects that can be troublesome to the patient with neuropathic pain. These include hypotension, bradycardia, and sedation (3). The systemic use of clonidine for neuropathic pain has been limited by these side effects to doses that are effective locally only when applied topically (4). Spinally administered clonidine is more effective than morphine in delaying the development of thermal hyperalgesia in rats with chronic constriction injury (CCI) of the sciatic nerve (5). Epidural and intrathecal clonidine are effective in the treatment of reflex sympathetic dystrophy (6), but doses that are effective by spinal administration also cause significant hypotension, bradycardia, and sedation (7).

Tizanidine hydrochloride (Zanaflex™; Elan Pharmaceuticals, South San Francisco, CA) is a novel centrally acting α_2 agonist that is currently available in an oral formulation for the treatment of muscle spasms. Tizanidine, like clonidine, is an agonist of the α_2 receptor in the spinal cord (8). Tizanidine has significant antinociceptive effects without hypotension in rats when given intrathecally (9,10) or subcutaneously (11). Although tizanidine is associated with a moderate incidence of sedation when administered at effective systemic doses, it is well tolerated clinically, with a small incidence of hemodynamic side effects (12).

No studies have evaluated the effectiveness of the systemic administration of tizanidine for the treatment of thermal hyperalgesia in an experimental neuropathic pain model. Although thermal hyperalgesia seems to be sympathetically mediated, there is little evidence that mechanical allodynia is relieved by treatments that decrease sympathetic function. IV clonidine dramatically decreases thermal hyperalgesia in the chronic CCI model of neuropathic pain, with only a moderate effect on mechanical hyperalgesia (13). This suggests that clonidine, and by extrapolation, tizanidine, would be more effective in treating thermal hyperalgesia because they both decrease central sympathetic outflow. Many patients with

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Address correspondence and reprint requests to Allen H. Hord, MD, Department of Anesthesiology, Emory University School of Medicine, 1364 Clifton Rd., N.E., Atlanta, GA 30322.

neuropathic pain present with both thermal hyperalgesia and mechanical allodynia. Given the limited armamentarium of treatments for neuropathic pain, symptom-specific treatments would be useful.

If systemic tizanidine is shown to be effective in treating neuropathic pain, it could be a better tolerated and a more practical alternative to either α -adrenergic antagonists or clonidine. The objective of this study was to determine whether systemic tizanidine relieves thermal hyperalgesia in rats with neuropathic pain caused by CCI of the sciatic nerve (14).

Methods

The Institutional Animal Care and Use Committee of Emory University approved this study. Male Sprague-Dawley rats weighing 250 to 300 g were used. Animals were housed in clear plastic cages with solid floors and soft, loose, absorbent bedding. They were allowed free access to food and water. The animals were housed in groups of two or three.

Animals were anesthetized with 40 mg/kg intraperitoneal (IP) pentobarbital. Subsequent doses of pentobarbital 2 to 4 mg/kg were administered as necessary to maintain adequate anesthetic depth. After an adequate depth of anesthesia was verified by lack of response to tail pinch, an incision was made from the left sciatic notch to the distal thigh. The subcutaneous tissue was bluntly dissected under the skin to expose the biceps femoris muscle. A cut was made into the muscle at the sciatic notch, and the muscle fibers were bluntly spread. Mayo scissors were placed through the muscle and a 2-cm cut in the muscle made toward the knee. By using blunt dissection, the muscle was separated from the layer below. A self-retaining retractor was placed to maintain exposure of the sciatic nerve. The sciatic nerve was freed from its investing fascia. With microinstruments, ligatures (4-0 chromic) were placed around the sciatic nerve and tightened until the suture barely indented the nerve under 40 \times magnification. Four ligatures were placed approximately 1 mm apart. The muscle was then closed with 4-0 Vicryl, and the skin was closed with steel clips. Identical surgery was then performed on the opposite (right) side, except that the ligatures were not placed (sham surgery). This was done to create an identical degree of muscle injury on both sides to ensure that the ability to respond to noxious stimulus was not altered by the surgery.

Forty-eight animals (Experimental Set 1) were placed in a Plexiglas cage with air holes on a glass testing table in which they could move freely (e.g., make postural adjustments), and they were allowed to acclimate to the environment for 10 min. The device (Ugo Basile, Milan, Italy) used for measurement of paw withdrawal latency (PWL) was similar to that

described by Hargreaves et al. (15). It consisted of a radiant heat source (high-intensity projector lamp bulb) located below the glass floor and projected through a round aperture. A photoelectric cell detected light reflected off the paw and turned off the lamp and electronic clock when withdrawal of the paw occurred. Tests were performed 3 days before surgery and then as described after surgery. Five sets of tests were done on each hind paw at each time of measurement and in random order. A minimum rest period of 2 min was allowed between each measurement on each paw. All of the postoperative tests were compared with the grand mean of the three preoperative tests (Time 0) for data analysis. The observer performing the testing was blinded as to treatment.

After three preoperative baseline testing periods on different days, rats underwent surgery as described previously. Thermal hyperalgesia was verified through PWL testing on postoperative Day 4. Only those 48 rats developing thermal hyperalgesia, defined as a >20% decrease in PWL on the left side compared with the right, underwent further testing and treatment. After the presence of thermal hyperalgesia was verified, 12 animals were randomly assigned to each of four treatment groups. On postoperative Days 4, 5, and 6, the rats were injected with 1 mL/kg of an IP blinded solution containing tizanidine 0.5, 1, or 2 mg/mL or normal saline according to randomized group assignment. Thirty minutes after dosing, the rats were again tested for PWL. The testing and treatment schemes were repeated on postoperative Days 5 and 6. On postoperative Day 9, 3 days after the third treatment, the rats were tested for PWL one final time.

Animals were placed in a Plexiglas cage with air holes in which they could move freely and were left to acclimate to the environment for 5 min. They were then monitored for 25 min. An array of 12 photoelectric cells was used to measure both ambulatory movement (i.e., breaking two adjacent photoelectric beams in succession) and total movement (i.e., breaking of any one photoelectric beam, e.g., scratching or ambulation).

A second group of 48 rats (Experimental Set 2) were individually placed in an activity cage (Opto-Varimex Mini; Columbus Instruments, Columbus, OH) that measured both ambulatory and total movement. The rats were monitored for 25 min after 5 min of acclimation to the environment as described previously. After this, animals were randomly divided into groups of 12 and treated with 1 mL/kg IP of a solution containing tizanidine 0.5, 1, or 2 mg/mL or normal saline. Investigators were blinded to the solution being administered. Thirty minutes later, the rats were placed back in the activity cage for 30 min.

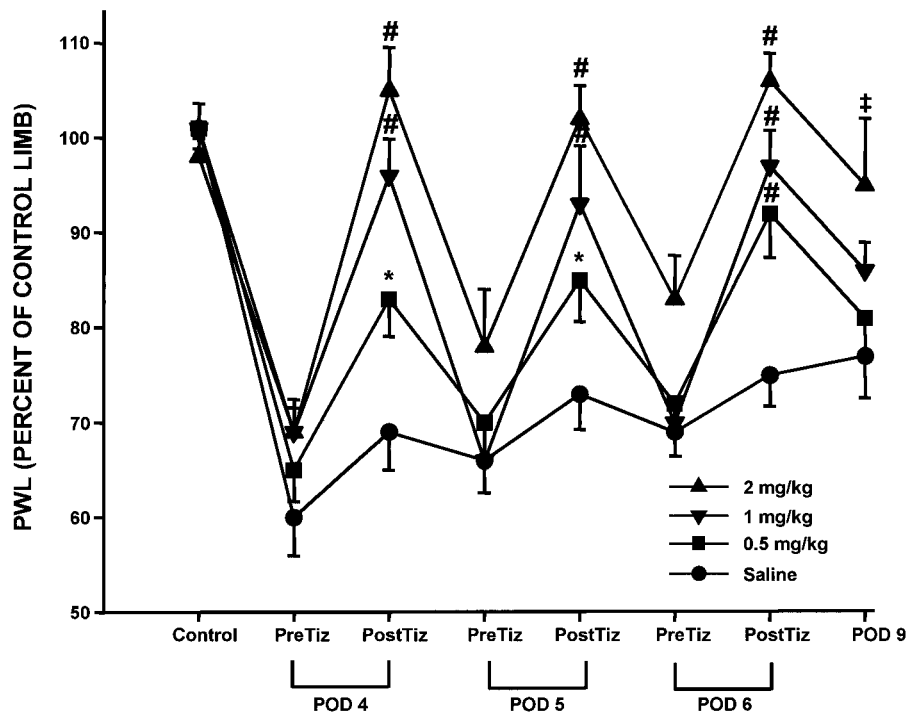


Figure 1. Tizanidine attenuates the thermal hyperalgesia associated with chronic constriction injury (CCI). Paw withdrawal latencies (PWLs) are plotted as a function of time and dose. All values are expressed as a percentage of the control (sham) side. All doses produced a rapid and significant reduction in the thermal hyperalgesia associated with CCI as compared with the pretreatment values (marked with # or *). However, the 0.5 mg/kg dose did not completely reverse thermal hyperalgesia (*). On postoperative days (PODs) 4, 5, and 6, tizanidine (Tiz) 1 and 2 mg/kg completely reversed hyperalgesia (#). Animals receiving a dose of 0.5 mg/kg also had complete reversal of the thermal hyperalgesia, but only on POD 6. On POD 9, animals previously treated with 2 mg/kg continued to have complete reversal of thermal hyperalgesia (‡). All data are mean \pm SEM for an *n* of 12 animals per group, with *P* < 0.05 considered significant.

Intra- and intergroup differences between preoperative and postoperative values and between Time 0 and subsequent measurement times were analyzed with a one-way analysis of variance for repeated measures followed by a *post hoc* Scheffé test for multiple comparisons. A *P* value of 0.05 was considered the minimum level for rejection of the null hypothesis.

Results

Left-sided (CCI) PWLs decreased significantly for all groups after surgery when compared with the right (Fig. 1). However, raw PWLs on the right (sham) side were not significantly changed from preoperative control values. This indicates no effect of surgery on the sham side. After the administration of tizanidine, but not saline, there was a significant improvement in PWL on postoperative Days 4 and 5 (*P* < 0.05) for all doses tested. Thermal hyperalgesia was completely reversed in the animals receiving either 1 or 2 mg/kg on postoperative Days 4, 5, and 6, as evidenced by PWL values that were not significantly different than preoperative control values. Although there was a significant (*P* < 0.05) improvement in PWL for the

animals receiving 0.5 mg/kg on postoperative Days 4 and 5, complete reversal of the thermal hyperalgesia was not evident until postoperative Day 6. On postoperative Day 9, only animals receiving 2 mg/kg had a PWL that was significantly different from saline (*P* < 0.05).

After the administration of saline, there were no changes in PWL on the sham side. However, after tizanidine, there were small increases in PWL on the sham side. These changes were significantly different on postoperative Day 5 after 0.5 mg/kg, on Days 4, 5, and 6 after 1 mg/kg, and on Days 4 and 5 after 2 mg/kg.

Some long-term effects of tizanidine administration were noted in the 2 mg/kg group when comparing pretreatment and posttreatment PWL values. On postoperative Days 5 and 6, the increase in pretizanidine PWL values was not statistically significant. However, on postoperative Day 9, the group that had received tizanidine 2 mg/kg on postoperative Days 4, 5, and 6 maintained complete reversal of thermal hyperalgesia and exhibited PWL values that were significantly higher than those in saline control animals (*P* < 0.05).

When compared with the saline control, both ambulatory and total movements were reduced after the

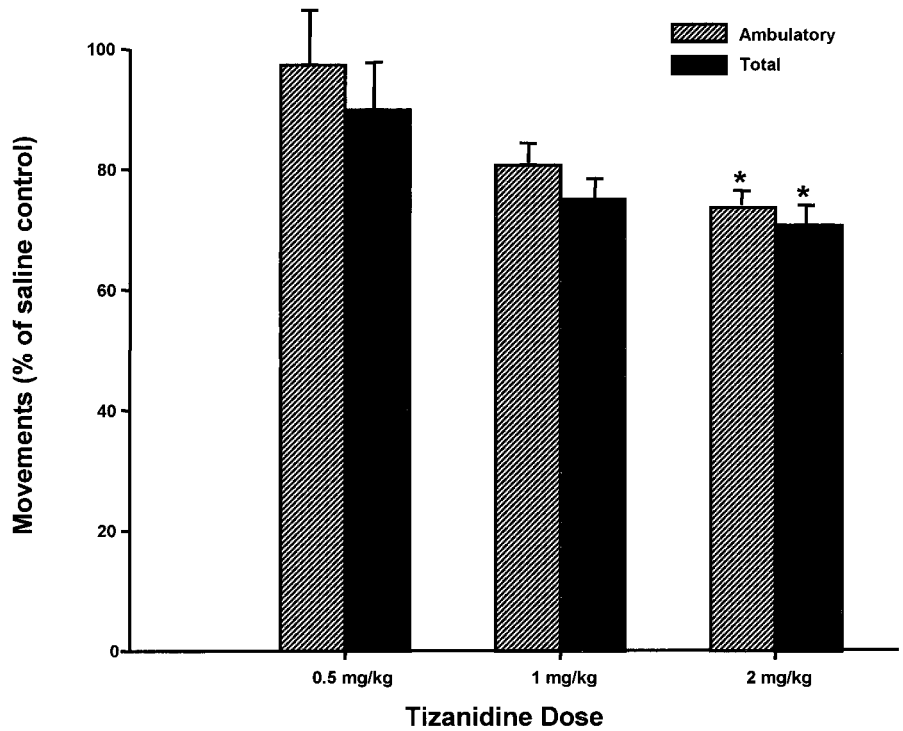


Figure 2. Tizanidine 2 mg/kg caused sedation, but neither 0.5 nor 1 mg/kg caused sedation. Ambulatory and total movements after tizanidine administration are expressed as a percentage of pretreatment values and normalized to saline control. Tizanidine 0.5 and 1 mg/kg did not significantly reduce the number of movements compared with saline control. However, tizanidine 2 mg/kg reduced both ambulatory and total movements (*), indicating the presence of sedation in these animals. Data are mean \pm SEM for an *n* of 12 animals per group, with *P* < 0.05 considered significant.

administration of tizanidine 2 mg/kg (Fig. 2). This indicated that tizanidine 2 mg/kg caused sedation in the rat. However, the administration of tizanidine 0.5 or 1 mg/kg did not significantly reduce movement of the animals compared with saline, indicating no sedation.

Discussion

Clonidine is often prescribed for patients with neuropathic pain. Its major drawback is that it may cause hypotension, bradycardia, and sedation at therapeutic doses (3). It has been suggested that tizanidine may also be useful for controlling chronic neuropathic pain (16). Leiphart et al. (17) reported that intrathecal tizanidine showed efficacy in a rat model of chronic neuropathic pain. In humans, tizanidine has been effective in the treatment of pain caused by tension-type headache and lower back pain (18-20). Tizanidine decreases excitatory transmission and facilitates segmental inhibition of neurons in animals and to temporarily reduce the incidence of painful paroxysms in patients with refractory trigeminal neuralgia (21). A potential advantage of tizanidine over clonidine in the treatment of neuropathic pain is that tizanidine is well tolerated in patients at therapeutic systemic doses (12). Sedation and dry mouth are frequent side effects but infrequently lead to discontinuation of therapy (22). Whereas postural hypotension is a limiting factor in clonidine use (23), in randomized studies there were

no differences in average blood pressure in tizanidine groups compared with placebo (12,22,24).

The Sprague-Dawley rat model used in this study was an effective model for thermal hyperalgesia, as evidenced by the significant decrease in PWL after surgery (*P* < 0.05). Once thermal hyperalgesia was surgically induced, tizanidine, administered on postoperative Days 4, 5, and 6, attenuated the thermal hyperalgesia in a dose-dependent manner. Whereas the 1 and 2 mg/kg doses of tizanidine completely reversed thermal hyperalgesia on postoperative Days 4, 5 and 6, only the 2 mg/kg dose effected long-term complete reversal as measured on postoperative Day 9. At the same time, only the 2.0 mg/kg dose caused sedation as measured by ambulatory and total movement. Tizanidine had a minor nonspecific analgesic effect on the sham side, which was inconsistent even at the largest dose.

It is possible that random fluctuations or gradual improvement in PWLs occurred during the postoperative period, accounting for the improvement that was seen in this study. However, maximum thermal hyperalgesia occurs between 4 and 10 days after CCI and is stable during that period (25). This study was designed to correspond with this narrow window when PWLs are stable.

After CCI, paw temperature initially increases, then decreases compared with the control limb (25). These changes are similar to those that have been anecdotally reported in patients with reflex sympathetic dystrophy (26). The starting temperature of the paw could

effect PWL by altering the time required to reach the critical threshold temperature required for nociceptor activation and paw withdrawal to occur. If the paw is warmer after CCI than the control paw, then it may require less time for the heat lamp to raise paw temperature to the nociceptive threshold. However, no statistically significant difference in paw temperatures occurs between Days 4 and 8 after CCI (25). There are significant changes in temperature that occur 10 days after CCI, but these are out of the time range of this study.

The activity cage records the number of movements made by the rats during the specified time period. This method for measurement of sedation has been used in many studies. Specifically, it has been shown to measure sedation in the presence of drugs not known to cause motor impairment (27,28). Tizanidine was sedating in other studies in the rat at doses as small as 1 mg/kg IP (29), which is consistent with the activity cage data that we have reported. However, it is possible that both activity cage and PWL data are altered by the ability of tizanidine to inhibit motor activity. Tizanidine has myotonolytic activity in a number of animal models (29). The restraint of movement imposed by spastic muscles and clonus is intimately related to enhanced muscle stretch reflex activity. Tizanidine, in animals and humans, modifies the phasic component of stretch reflex activity. Doses used in this study are well above the minimum doses exhibiting myotonolytic activity in the rat. Inhibition of motor function could decrease movement or increase PWLs. Antinociceptive efficacy has been shown with doses of tizanidine considerably smaller than those that caused motor incoordination (30). Kameyama et al. (31) have shown that the 50% effective concentration of tizanidine is 1.79 $\mu\text{mol/kg}$ subcutaneously in mice. This dose corresponds to the 0.5 mg/kg dose used in our study. They also found sedation (decreased wheel-revolving activity) at 5.51 $\mu\text{mol/kg}$, which is similar to the 2 mg/kg (6.8 $\mu\text{mol/kg}$) dose, which we likewise found sedating. In contrast, the dose causing motor incoordination was 148.2 $\mu\text{mol/kg}$, which is 21 times the largest dose used in our study. We observed no difficulty in paw withdrawal during this study. Although it was not measured, the speed of the withdrawal motion seemed normal. Therefore, we do not think that inhibition of motor function affected the PWLs seen in this study.

The mechanism of the antinociceptive effect of tizanidine is not clear. Iontophoretic application of tizanidine to laminae II and III of the cat causes a reduction in responses of laminae IV and V neurons evoked by noxious stimuli (32). Spontaneous firing was also depressed, but responses to innocuous stimuli were unaffected. In contrast, γ -aminobutyric acid application resulted in a reduction in response

to both noxious and innocuous stimuli. The IV administration of tizanidine caused the same reduction in response to noxious stimuli (32). It is believed that tizanidine's antinociceptive properties are caused by selective interference with excitatory amino acid release, including *N*-methyl-D-aspartate (32-34).

Further information on the profile of tizanidine in neuropathic pain could have been gained by including measures of mechanical allodynia. We used the CCI model described by Bennett and Xie (14) because it has pronounced thermal hyperalgesia that is completely reversible by chemical sympathetic blockade (35). We chose to measure thermal hyperalgesia because we believed that a drug such as tizanidine, which decreases sympathetic outflow, would most likely improve it. Kim et al. (36) investigated the effect of sympathectomy on mechanical allodynia in the L5 and L6 nerve root ligation model. They found that mechanical allodynia was relieved by postoperative sympathectomy and prevented by preoperative sympathectomy. However, Ringkamp et al. (37,38) found that neither surgical sympathectomy nor $\alpha 1$ or $\alpha 2$ receptor blockade altered mechanical allodynia in an L5 spinal nerve ligation model. In the partial sciatic nerve ligation model, Shir and Seltzer (39) found that sympathectomy at the time of nerve injury aggravated mechanical allodynia. Therefore, we did not expect that mechanical allodynia would improve after tizanidine. In effect, we have reported information that was preselected to be positive; we did not perform tests we expected to be negative. Our findings suggest that tizanidine may have promise in the treatment of neuropathic pain syndromes that are sympathetically mediated or of patients with thermal hyperalgesia. These findings should not be extrapolated to assume that tizanidine improves all types of neuropathic pain, including mechanical allodynia.

A recent preliminary report of a retrospective assessment of response to oral tizanidine suggested that tizanidine was well tolerated and effective in 71% of complex regional pain syndrome I and II patients, although it was less effective for other types of neuropathic pain (40). Further research is warranted to determine the efficacy and tolerability of tizanidine in patients with chronic neuropathic pain, including complex regional pain syndrome I and II. In addition, comparison should be made with clonidine in patients to determine whether, in fact, pain relief is similar with fewer side effects.

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