Animal Study

Tianeptine Reduces Mechanical Allodynia in Spinal Nerve-ligated and Chemotherapy-induced Neuropathic Mice

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Background: Spinal nerve-ligated neuropathy and chemotherapy-induced neuropathy produce a persistent tactile allodynia in mice. Tianeptine is an antidepressant that exhibits structural similarities to tricyclic antidepressants but has distinct neurochemical properties.

Objective: Here we examined the effects of intraperitoneal (i.p.) tianeptine on allodynia in spinal nerve-ligated and chemotherapy-induced neuropathic mice.

Study Design: A randomized, experimental trial.

Setting: Laboratory animal study.

Methods: Spinal nerve-ligated neuropathy was induced in a Chung model made by ligating the L5 spinal nerve. Chemotherapy-induced neuropathy was induced by injecting vincristine (0.1 mg/kg/day; i.p.) on the following schedule: 5 days on, 2 days off, for14 days. Tianeptine (10, 30, and 50 mg/kg) and saline were administered, respectively, to both groups of neuropathic mice (n = 5 for each group). We evaluated mechanical allodynia using von Frey hairs prior to drug injections and at 30, 60, 90, 120, 180, and 240 minutes, and 24 hours after injections. We also measured the changes in activate transcription factor 3 (ATF3) level in the dorsal root ganglion (DRG) in each group in order to understand the analgesic mechanism of tianeptine.

Results: Both spinal nerve-ligated and chemotherapy-induced neuropathic mice showed prominent allodynia. The control group showed no differences in mechanically induced allodynia compared to the experimental groups. For the tianeptine groups, paw-withdrawal thresholds in response to mechanical stimuli were significantly lower than the pre-administration values and values from the control group ($P < 0.05$). The increase in DRG ATF3 in neuropathic mice was reduced by tianeptine ($P < 0.05$).

Limitations: Less is known about the transcription factors that affect inflammation signaling.

Conclusions: Tianeptine administered i.p. reduces mechanical allodynia in spinal nerve-ligated and chemotherapy-induced neuropathic mice models. These effects were confirmed by attenuation of previously increased DRG ATF3.

Key words: Tianeptine, spinal-nerve ligation, chemotherapy-induced neuropathic, activating transcription factor 3

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The International Association for the Study of Pain (IASP) has reported that neuropathic pain (NP) may be caused by a variety of lesions or diseases of both the peripheral and central nervous system (1). NP has been documented to affect around 6% – 8% of the general population and is associated with a decreased quality of life (2,3). Various classes of drugs have been shown to have efficacy against

NP, such as opioids, topical lidocaine, capsaicin, antiepileptic drugs, antidepressants, etc. (4-7). Among antidepressants, tricyclic antidepressants (TCAs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) have been investigated experimentally and clinically for their ability to manage neuropathic pain (8-10).

Tianeptine is a kind of antidepressant with structural similarities to TCAs, but it selectively enhances serotonin reuptake (11). Compared to TCAs and SNRIs, there are only a few published studies about the analgesic effects of tianeptine. Intrathecal administration of tianeptine has been found to induce analgesia in a rat model of neuropathic pain (12), as well as in an inflammatory pain model (13). Additionally, intravenous tianeptine has an antinociceptive effect in a rat model of visceral pain (14). Nevertheless, there are no known reports on the effect of tianeptine on chemotherapy-induced neuropathy in animals or humans. Moreover, the analgesic mechanism of tianeptine has not been thoroughly investigated.

The main objective of this study is to evaluate the analgesic effect of tianeptine on spinal nerve-ligated and chemotherapy-induced neuropathy in mice with persistent tactile allodynia. Also, we examined the analgesic mechanisms of tianeptine with respect to changes in activating transcription factor 3 (ATF3) in the dorsal root ganglion (DRG).

Methods

Animals and Preparation

This protocol was approved by the Institutional Animal Care and Use Committee at the Catholic University of Seoul, Korea. Experiments were conducted with wild-type male C57BL/6 mice weighing 25 – 30 g. The mice were housed individually in standard cages with soft bedding. They were housed under an alternating 12/12-hour light/dark cycle in a temperature-controlled room (22°C ± 0.5°C). Food and water were freely available. The mice were anesthetized with isoflurane under spontaneous respiration. Spinal nerve-ligated neuropathy was created by tightly ligating the L5 spinal nerve according to the method described by Kim and Chung (15). Animals displaying a 50% withdrawal threshold < 0.6g by postoperative day 7 were defined as the neuropathic model (16). Chemotherapy-induced neuropathy was produced by injecting vincristine (0.1 mg/kg/day, intraperitoneally [i.p.]) using a 5-day-on, 2-day-off schedule for 14 days, according to a method described by Uceyler et al (17).

Tianeptine Treatment

Tianeptine (Jeil, Seoul, Korea) and saline were administered i.p., respectively, to the spinal nerve-ligated and chemotherapy-induced neuropathy mice in both groups. Tianeptine was dissolved in 0.9% saline, and i.p. injected 10, 30, and 50 mg/kg. Tianeptine-treated mice showed no impairment in mobility or motor function over the course of this experiment. Motor function was measured using an accelerating rotarod test (data not shown). The rotarod test was performed according to a method described by a previous study (18).

Behavioral Tests

We evaluated mechanical allodynia in mice using von Frey filaments prior to drug administration and then again at 30, 60, 90, 120, 180, 240 minutes, and 24 hours after drug administration. Animals were placed on an elevated wire cage. After giving the mice 30 minutes to adapt to the testing environment, we stimulated the plantar surface of the hind paw with von Frey filaments (also known as Semmes-Weinstein filaments, Stoelting, Wood Dale, IL, USA, 2.44 – 4.31g), using the up-down method (19). Each von Frey filament was pressed perpendicularly against the mid-plantar surface of the hind paw from below the wire floor and held in place for 6 – 8 seconds in a slightly bent form. Flinching of the paw was designated as a positive response, as we have documented in a previous study (20). Results were tabulated, and the 50% response threshold was computed using a previously published formula (21,22). When the tactile threshold fell to approximately ≤ 0.6 g, we defined the animal as having tactile allodynia. Mobility and motor function changes in the neuropathic rats were evaluated by rotarod testing (Acceler rota-rod for rats 7750; Ugo Basile, Comerio-Varese, Italy). The neuropathic rats were acclimatized to the revolving drums, and they were habituated to handling to ameliorate any stress during testing. The rats were given 3 training trials on the revolving drums (10 – 15 rpm) for 2 days before the actual day of testing. The rats that were able to remain on the revolving drum for a minimum of 150 seconds were selected for drug testing. The mean of 3 training runs served as a control performance time. The rotarod performance time was measured at 30, 60, 90, 120, 180, 240 minutes, and 24 hours after i.p. injection. Each test was performed 3 times at 5-minute intervals, and the mean values were compared (18).

Tissue Harvesting and Preparation

Thirty minutes after tianeptine or saline injection, the animals were anesthetized with Euthasol and intracardiac perfusion was performed with 0.9% saline, followed by 4% paraformaldehyde. For immunohistochemistry, the L5 DRG on the ligated side was dissected. DRGs were fixed in 4% paraformaldehyde (pH 7.4) and cryoprotected in 30% sucrose. DRGs were mounted on glass slides.

Immunohistochemistry

Fixed tissue was then embedded for sectioning and processed using common immunohistochemical methodologies (23). DRG tissues were sectioned at 10-µm thickness for examination. We obtained 2 DRG sections each from 4 control group and 6 experimental group animals. The primary antiserum we used was rabbit anti-ATF3. Binding sites appeared with anti-rabbit IgG antibodies conjugated with Alexa-488 (1:500; Invitrogen, Carlsbad, CA, USA). Nuclei were counter stained using ToPro3 (1:500; Invitrogen). All images were captured by a Leica TCS SP5 confocal imaging system and quantified using Image-Pro Plus v.5.1 software. ATF3 staining was quantified by measuring the total integrated intensity of pixels divided by the total number of pixels in a standardized area with 4 to 6 mice. ATF3 data are presented as percentage change from the corresponding control group.

Statistical Analysis

Data are expressed as mean ± SE. For comparisons between time courses and tianeptine doses regarding paw withdrawal threshold (PWT), 2-way ANOVA was used with a Bonferoni post-hoc correction for multiple group comparisons. For comparison of ATF3 changes, a t-test was used. A P value of < 0.05 was considered significant.

Results

Both spinal nerve-ligated and chemotherapy-induced neuropathic mice showed a prominent allodynia ($P < 0.05$, Fig. 1). None of the experimental mice experienced any side effects such as dizziness or sleepiness and motor weakness was not observed on the rotarod test.

Tianeptine Reduced Allodynia in Spinal Nerve-ligated Mice

The control group had no differences regarding mechanical allodynia from the mono-neuropathic mice ($P > 0.05$; Fig. 2A). In spinal nerve-ligated mice, the group that received 10 mg/kg group of tianeptine (i.p.) showed an increase in the paw withdrawal threshold by 30 minutes after tianeptine injection compared to the saline group ($P < 0.05$; Fig. 2A). Among the mice groups that received 30 and 50 mg/kg of tianeptine, there were significant antiallodynic effects between 30 and 90 minutes and between 30 and 180 minutes, respectively, compared to the saline group ($P < 0.0001$; Fig. 2A).

Tianeptine Reduced Allodynia in Chemotherapy-induced Neuropathic Mice

The control group showed no significant difference in tactile hyperalgesia compared to the poly-neuropathic mice ($P > 0.05$; Fig. 2B). In vincristine-induced neuropathy, 10, 30, and 50 mg/kg of tianeptine significantly elevated paw withdrawal thresholds between 30 and 60 minutes, between 30 and 120, and between 30 and 240 minutes, respectively ($P < 0.0001$; Fig. 2B). The vincristine-induced neuropathic pain model showed a better effect than the spinal nerve-ligated neuropathic pain model when the same dose of tianeptine was used ($P = 0.0433$; Fig. 2C).

ATF3 Decreased in DRG after Tianeptine Treatment in Spinal Nerve-ligated and Chemotherapy-induced Neuropathic Mice

Immunohistochemistry showed that ATF3 binding components increased in spinal nerve-ligated and chemotherapy-induced neuropathic mice (representative of both control groups shown in Figs. 3A and 3D). Spinal nerve-ligated DRG showed a larger increase in ATF3 than did chemotherapy-induced mice ($P < 0.01$; Fig 3B, 3E). ATF3 was lower in both groups of mice than those receiving tianeptine treatment ($P < 0.0001$; Fig 3C, 3F). The increased immunoreactivity of ATF3 was abolished after tianeptine treatment in both mono-neuropathic and poly-neuropathic DRGs ($P < 0.001$; Fig 4).

Discussion

This study found that tianeptine administered intraperitoneally decreased mechanical allodynia in spinal nerve-ligated and chemotherapy-induced neuropathic mice. We also found that the ATF3 level was significantly reduced in both types of neuropathic DRGs after tianeptine injection. These results suggest that tianeptine plays a significant analgesic role by decreasing ATF3 level in the dorsal horn of the spinal cord.

Our results are consistent with those of an earlier

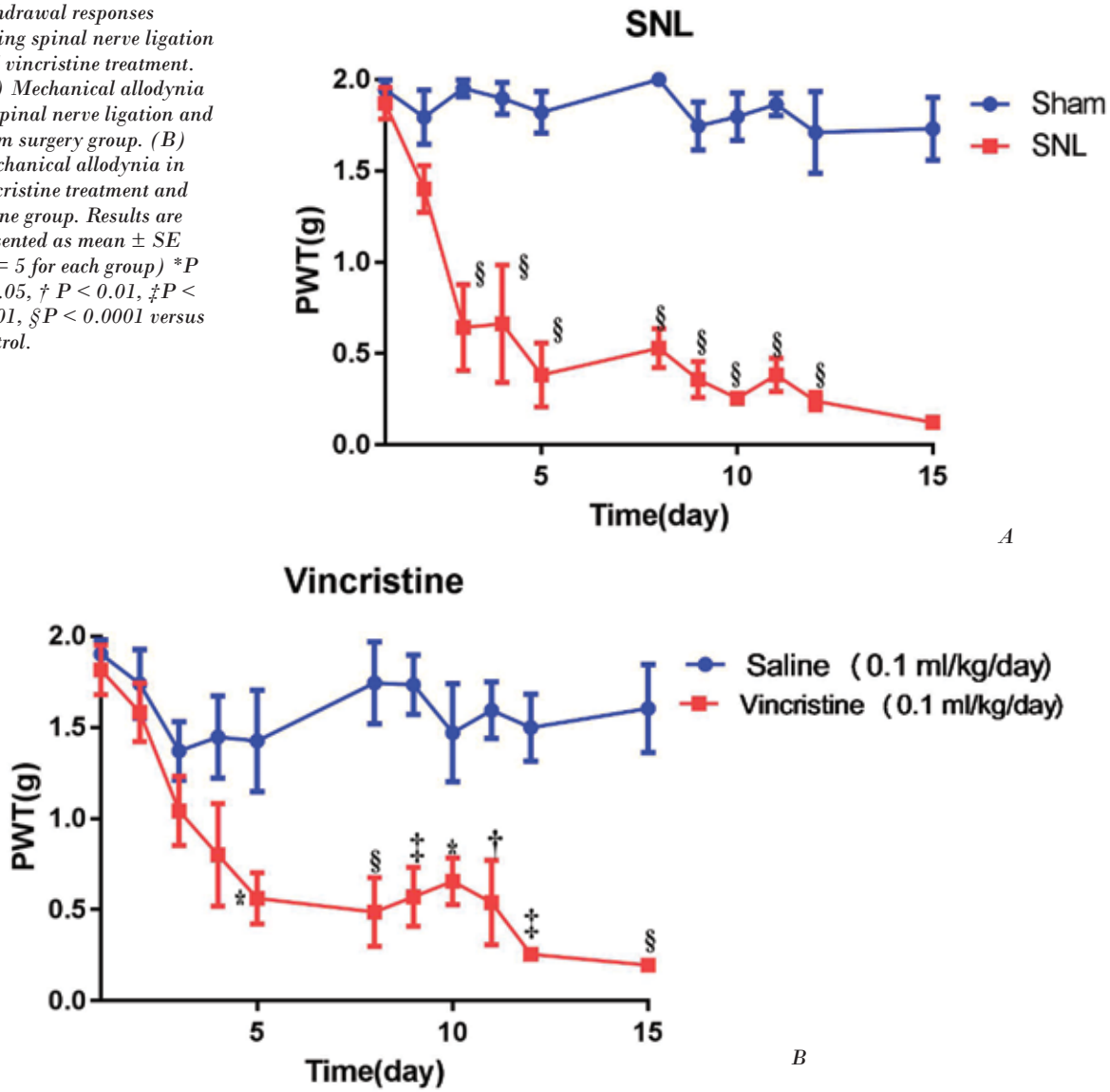
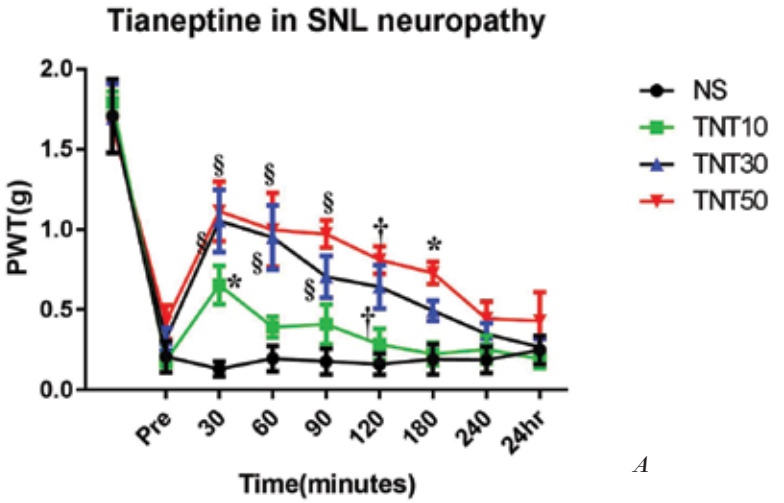


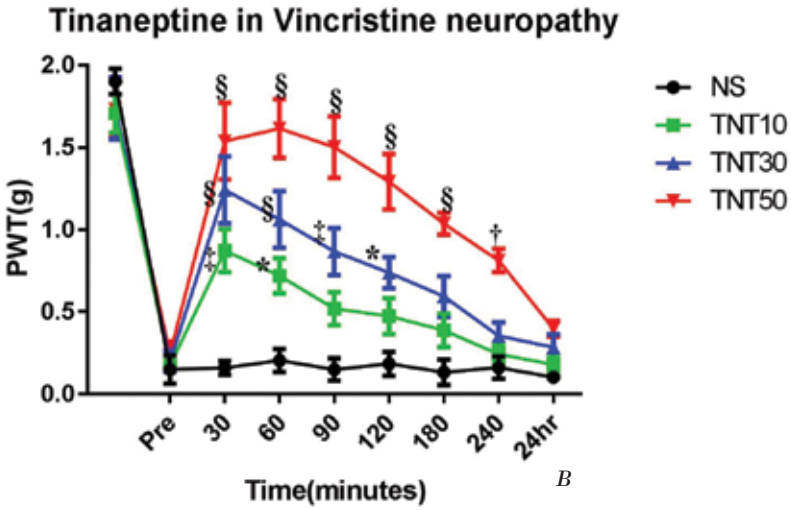
Fig. 1. *Changes of withdrawal responses during spinal nerve ligation and vincristine treatment. (A) Mechanical allodynia in spinal nerve ligation and sham surgery group. (B) Mechanical allodynia in vincristine treatment and saline group. Results are presented as mean ± SE (n = 5 for each group) *P < 0.05, † P < 0.01, ‡P < 0.001, §P < 0.0001 versus control.*

study in which tianeptine was found to have an analgesic effect in mice (14). That study also found that intrathecally administered tianeptine increased 5-HT and NE levels in the dorsal horn of the spinal cord (12). Uzbay et al (14) found that acute administration of tianeptine induced a dose-dependent antinociceptive effect in mice subjected to both tail-flick and hot-plate tests. Additionally, intrathecally administered tianeptine may be useful as a therapeutic drug for managing inflammatory pain, according to a previous study (13). These findings are similar to those of a previous rat model study. Nevertheless, in the present study, tianeptine was injected i.p. at various doses in spinal nerve-ligated and chemotherapy-induced neuropathic mice; compared to saline injected mice, tianeptine injected mice had significantly decreased reactions to the von Frey filament test and decreased ATF3 level according to immunohistochemical staining.

Cyclooxygenases and prostaglandin (PG) play decisive roles in inflammatory activity (24). Several studies

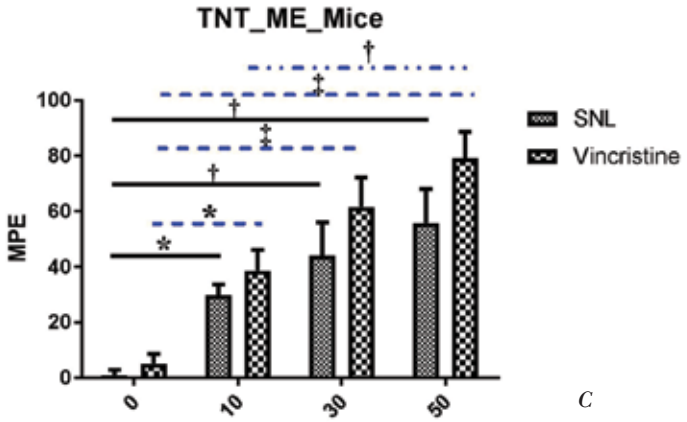


A



B

Fig. 2. *Tianeptine reduces allodynia in neuropathic mice. A: Spinal nerve-ligated mice were injected with tianeptine and saline at 10-, 30-, 50-mg/kg doses (n = 5, for each group). Paw withdrawal threshold in response to mechanical stimuli was significantly reduced in post-injection tests compared to the pre-injection tests and compared to the control group. B: Chemotherapy-induced mice were injected with tianeptine and saline at 10-, 30-, 50-mg/kg doses (n = 5, for each group). Paw withdrawal thresholds were significantly different. C: Paw withdrawal threshold decreased as the dose of tianeptine increased. ME; Maximal Effect. *P < 0.05, † P < 0.01, ‡P < 0.001, §P < 0.0001.*



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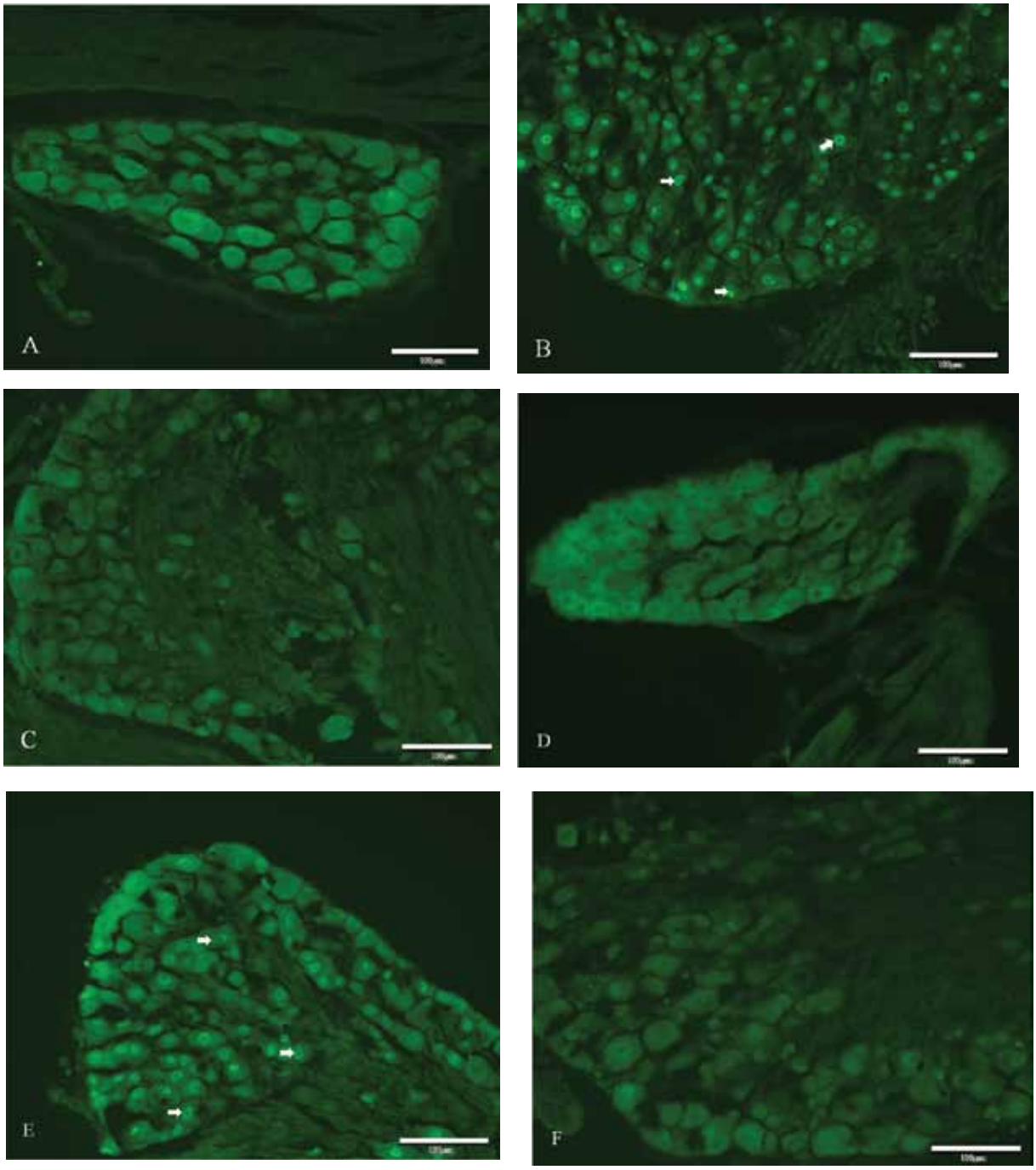

Fig. 3. *Immunohistochemistry showed that ATF3 binding components were elevated in spinal nerve-ligated and chemotherapy-induced neuropathic mice. We obtained 2 DRG sections each from 4 control group and 6 experimental group animals. Effects of i.p. tianeptine on ATF3 after spinal nerve-ligated and chemotherapy-induced neuropathy. A: Representative DRG stained for ATF3 in a mouse with sham surgery. B: Representative DRG stained for ATF3 in a spinal nerve-ligated mouse with i.p. saline. C: Representative DRG stained for ATF3 in a spinal nerve-ligated mouse with i.p. tianeptine. D: Representative DRG stained for ATF3 in a control mouse. E: Representative DRG stained for ATF3 in a vincristine-treated mouse with i.p. saline. F: Representative DRG stained for ATF3 in a vincristine-treated mouse with i.p. tianeptine.*

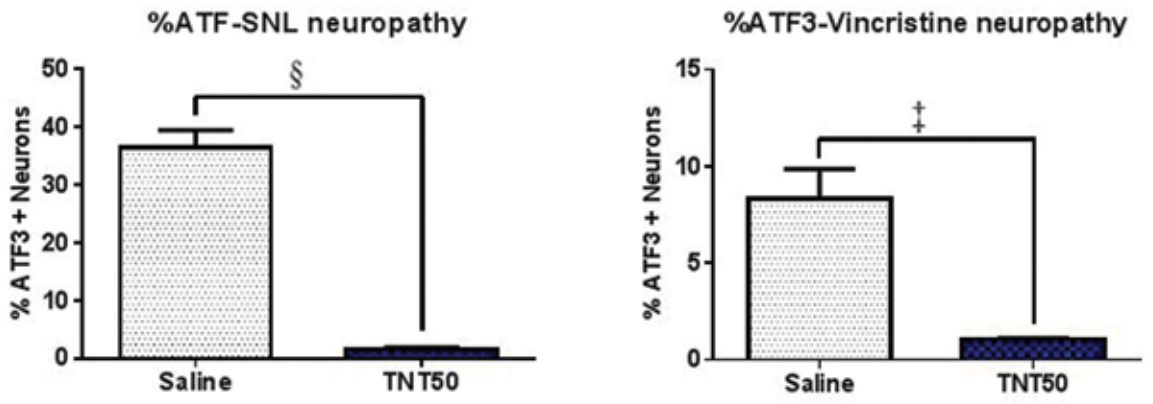

Fig. 4. *The percentage of ATF3 in DRG neurons was significantly lower in tianeptine-injected mice compared to saline-injected mice in both neuropathic mouse models.* $‡P < 0.001$, $§P < 0.0001$.

have documented that many transcription factors, including NF-kB, Ap-1, Sp1, and C/EBP, initiate PG-2 in inflammatory signaling (25). However, less is known about the transcription factors that affect PG-2 signaling. Hellmann et al (26) have shown that, during acute inflammation, ATF3 negatively regulated PG-2, indicating that ATF3 is a factor affecting the inflammatory cascade. ATF3 has also been proposed to be a nerve injury marker in the DRG. As a transcription factor, ATF3 is thought to play a critical role in regulating the signaling that leads to impairment of sensory afferent function (27). ATF3 is induced by several stress signals, including ischemia and oxidative stress, according to some in vivo studies (27,28). Furthermore, increased ATF3 was observed in spinal nerve-ligated mice models with chemotherapy-induced polyneuropathy and nerve injury (20,29). Similar results were observed with injection of vincristine in a rat model. Herein, we reported on ATF3 level according to immunohistochemistry using ToPro3 staining (fluorescent green), which was recognized according to an intense fluorescent mark in the nuclei region. We demonstrated that ATF3 decreased significantly in tianeptine-treated mice. Opioid receptors are also present in the DRG and may be its relationship to ATF3 in neuropathic pain model (30). Recently, tianeptine has been characterized as a μ-opioid receptor (MOR) agonist (31). Tianeptine showed full agonist at MOR, also inhibition of cAMP accumulation. The previous study demonstrated that tianeptine's modulation of the glutamatergic system may occur via activation of opioid signaling for analgesic effect (31). Furthermore, the present study showed tianeptine decreased the concentration amount of ATF3.

Adverse effects of tianeptine are similar to those of other antidepressants. The adverse effects usually associated with tianeptine include gastrointestinal effects, such as nausea, constipation, abdominal pain, and central nervous system disturbances, like headache, dizziness, and sleepiness (32,33). We studied lethal doses of tianeptine in a pilot study wherein we administered 100 mg/kg of tianeptine; however, in that study, all of the mice died. When we reduced the tianeptine amount to 60 mg/kg, the mice showed signs of seizure-like movement. We determined that the effective maximal dose of tianeptine was 50 mg/kg, and this study showed an effective dose of tianeptine in neuropathic mice. However, there is a limitation to this study in that we only used the immunohistochemistry method and not protein expression.

Conclusion

In conclusion, our work suggests that i.p. tianeptine yields significant effects on mechanical allodynia in spinal nerve-ligated and chemotherapy-induced neuropathic mice. The effect of tianeptine to drive a significant decrease in ATF3 activation in the DRG may prove insightful for further in vitro studies of tianeptine treatment for neuropathic pain.

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