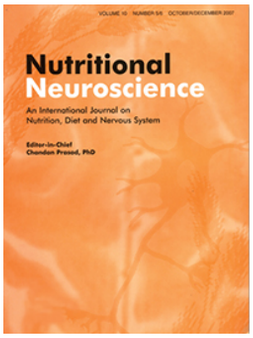

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Vitamin D supplementation attenuates the behavioral scores of neuropathic pain in rats

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Objective(s): Neuropathic pain due to lesion or dysfunction of the peripheral or central nervous system is often refractory to the conventional analgesics. Currently, there is no proven treatment to prevent or cure neuropathic pain. A recent surge of new data suggests the potential effects of vitamin D in the medical community. This study was designed to determine whether acute or chronic vitamin D administration was effective in alleviating symptoms of neuropathic pain in a rat model of neuropathic pain.
Materials and Methods: Neuropathic pain was induced by chronic constriction injury (CCI) of the sciatic nerve in the rats that resulted in thermal hyperalgesia, mechanical, and cold allodynia.
Results: Acute vitamin D injections (250, 500, and 1000 unit/kg i.p.) on the 7th, 14th, and 21st postoperative days could not attenuate mechanical and cold allodynia as well as heat hyperalgesia compared to CCI group. But when vitamin D (1000 unit/kg i.p.) administration was started on the first day after surgery and given daily until the 21st day, cold allodynia and heat hyperalgesia considerably were attenuated. However, no differences in paw withdrawal thresholds were observed.
Conclusion: These results indicate that chronic vitamin D administrations can attenuate the behavioral scores of neuropathic pain in rats.

Keywords: Vitamin D, Neuropathic pain, Rat, Hyperalgesia

Introduction

Neuropathic pain which is caused by primary or secondary damage of the nervous system characterizes by hyperalgesia, allodynia, and spontaneous pain.[1] There are multiple anatomical sites of lesions in the neuropathic pain.[2] In these patients, pain is usually chronic, spontaneous, and/or stimuli-evoked and sometimes difficult to treat.[3] Despite intensive research on the neurobiological mechanisms involved in this chronic pain, effective therapy and the underlying mechanisms are poorly detect.[4,5] The opioids in this pain state have limited efficacy as compared to other pain states.[6] Therefore, the development of more efficacious analgesics with improved side effect profiles is currently a major focus.[7]

Vitamin D is a fat-soluble secosteroid.[8] The emergence of new data suggests that the benefits of Vitamin D extend beyond healthy bones.[9] Recently, the relationship between pain and Vitamin D is investigated.[10] There are various forms of Vitamin D; the most common forms are VitaminD3 (cholecalciferol) and Vitamin D2 (ergocalciferol).[11] Vitamin D deficiency linked to a headache, abdominal, knee and back pain, and also with fibromyalgia.[12] Long-term Vitamin D deficiency weakens the immune system and leads to chronic inflammation.[13] Experimental studies showed that inflammation leads to the hyperalgesia.[14] Patients suffering from neuropathic pain usually experience other comorbidities such as sleep, anxiety, and mood disorders.[15] An adequate level of Vitamin D improves the quality of life.[16] Research in the area of chronic pain and Vitamin D deficiency remains limited. Thus, more research is necessary to determine whether Vitamin D is useful in the treatment of various types of pain. So this study was designed to determine whether acute or chronic Vitamin D administration was effective in attenuating hypersensitivity in a rat model of neuropathic pain.

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Material and methods

Animals and housing conditions

The experiments were performed on male Sprague–Dawley rats (200–250 g). They were housed four per cage, in a room under controlled temperature (23 ±

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2°C), humidity (50%), and lighting (12/12 h light/dark cycle), with food and water available *ad libitum*. All experiments were approved by the ethical committee of Shiraz University of Medical sciences international branches and followed the European Commission Directive (86/609/EEC) for animal experiments.

Chemicals

Vitamin D was obtained from Darupakhsh pharmaceutical Co., Iran. Acetone was bought from Iran Kave Co., Iran. Vitamin D was diluted in almond oil.

Neuropathic pain model

The rats were anesthetized with ketamine (50 mg/kg i.p.) and xylazine (10 mg/kg i.p.). The common sciatic nerve was exposed and dissected from surrounding connective tissue. Four ligatures (4.0 chromic Gut) were tied loosely around the common sciatic nerve with a 1–1.5 mm interval between ligatures. Sham-operated rats had the same surgery, the left common sciatic nerve was exposed but no ligation was made.[17] The rats were housed individually in cages after the surgery. All experiments followed the guide lines on the ethical standard for investigation of experimental pain in animals and were also approved by the Research.

Behavioral tests of neuropathic pain

Heat hyperalgesia, cold, and mechanical allodynia were determined by using the radiant heat plantar, acetone, and von Frey test, respectively, as the behavioral score of neuropathic pain. After cage exploration and major grooming activities ceased, the behavioral tests were done. The hypersensitivity to the neuropathic pain was determined 1 day before the surgery as the baseline value and also 45 min after the injections on the 7th, 10th, 14th, and 21st postoperative days.[18]

Thermal hyperalgesia (plantar test)

Paw withdrawal latency in response to radiant heat was measured by using plantar test apparatus (Ugo Basile, Varese, Italy). Thermal withdrawal latency was defined as the latency (seconds) between the heat stimulus onset and paw withdrawal using a feedback-controlled shut-down unit. A cut-off time of 22 s was used to avoid tissue damage. Mean latency of the withdrawal response for ipsilateral (operated) and contralateral (non-operated) paws was calculated separately.[19]

Cold allodynia (acetone test)

Cold allodynia was performed with using the acetone spray test (evaporation-evoked cooling) as described previously. The frequency of paw withdrawal was expressed as a percentage (the number of paw withdrawals/number of trials × 100).[20]

Mechanical allodynia (von Frey filament stimulation)

To examine mechanical allodynia, withdrawal threshold to mechanical stimuli was measured using von Frey filaments (steeling, Wood Dale, IL, USA) in the following order: 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 26.0 and 60 g.[21]

Treatment

Because common clinical practice indicates that neuroprotective interventions should be used immediately after the injury, it was further hypothesized that early vitamin D treatment would be more effective than treatment initiated after pain hypersensitivity has been established. To compare early versus late treatment, rats were given vitamin D, starting immediately after surgery and continued daily until the 21st day. The effects of acute injection of vitamin D on pain hypersensitivity were also tested at 45 min after administration, on the 7th, 14th, and 21st days after surgery.

Intermittent treatment

Rats were injected intraperitoneally with the vehicle and the baseline paw withdrawal thresholds were measured. Vitamin D (250, 500, and 1000 unit/kg i.p.) had been injected on the 7th, 14th, and 21st days after surgery, at 45 min before the behavioral tests.

Repeated treatment

Drug or vehicle was administered during the onset phase of model development. These times were chosen to clarify the possible effects of repeated vitamin D administration during the onset phase of the model. Vitamin D (250, 500, and 1000 unit/kg i.p.) was administered one day after surgery and continued once daily for 20 additional days. Behavioral tests were done on the 7th, 14th, and 21st days after surgery.[22]

The vehicle groups were given almond oil injections according to the same schedule.

For repeated treatment, injections were always given 45 min before measurement of paw withdrawal thresholds, withdrawal frequency, and paw withdrawal latency.[23]

Statistical analysis

Parametric data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post-hoc test for multiple comparisons. Time-course analysis of the behavioral data was compared by ANOVA repeated measures for heat hyperalgesia and cold allodynia tests.[24] Moreover, the nonparametric data resulted from von Frey test were converted to parametric data by rank transformation method and new data were analyzed by ANOVA.[25]

Results

Behavioral responses of chronic constriction injury model of neuropathic pain

Sciatica nerve ligation did not damage the motor activity and the rats seemed healthy. The appearance of the surgical paw slightly changed but this did not interfere with the normal activity of the rats.

The sciatic nerve ligation decreased paw withdrawal latency to the thermal stimulus significantly ($P < 0.001$), but the control group did not produce any significant change in the withdrawal latency (Fig. 1A).

Application of acetone to the medial surface of the nerve-ligated hind paw led to the significant rises in the withdrawal frequency of the chronic constriction injury (CCI) group compared to the control group Fig. 1B ($P < 0.001$). Also, Fig. 1C shows the results of the behavioral tests for the mechanical allodynia. Sciatic nerve ligation significantly enhanced the sensitivity of the ipsilateral hind paw to the mechanical

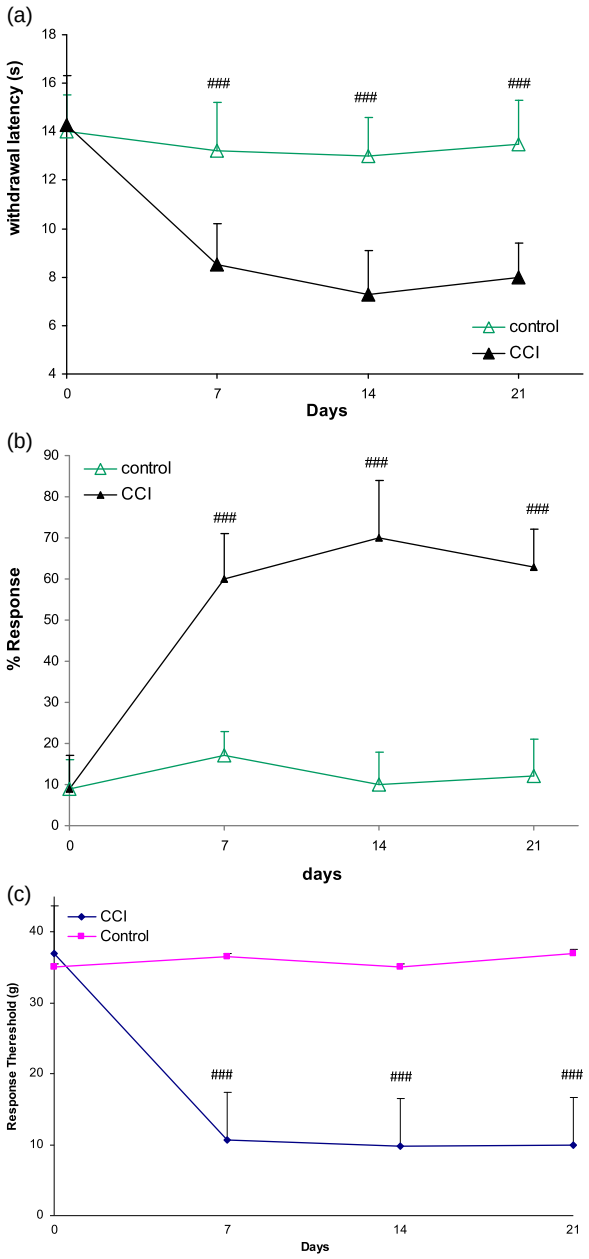

Figure 1 Behavioral responses of CCI model of the neuropathic pain. (A) The effects of CCI of the sciatic nerve on the heat hyperalgesia. (B) The effects of CCI of the sciatic nerve on the cold allodynia. (C) The effects of CCI of sciatic nerve on mechanical allodynia. The results are expressed as mean ± S.E.M., $n = 8$ in all groups. ###$P < 0.001$ versus CCI group. Results from von Frey test (Fig. 1C) are expressed as mean rank ± S.E.M. The vehicle groups (CCI) received almond oil according to the treatment schedule. Control group had the same surgery, the left common sciatic nerve was exposed but no ligation was made.

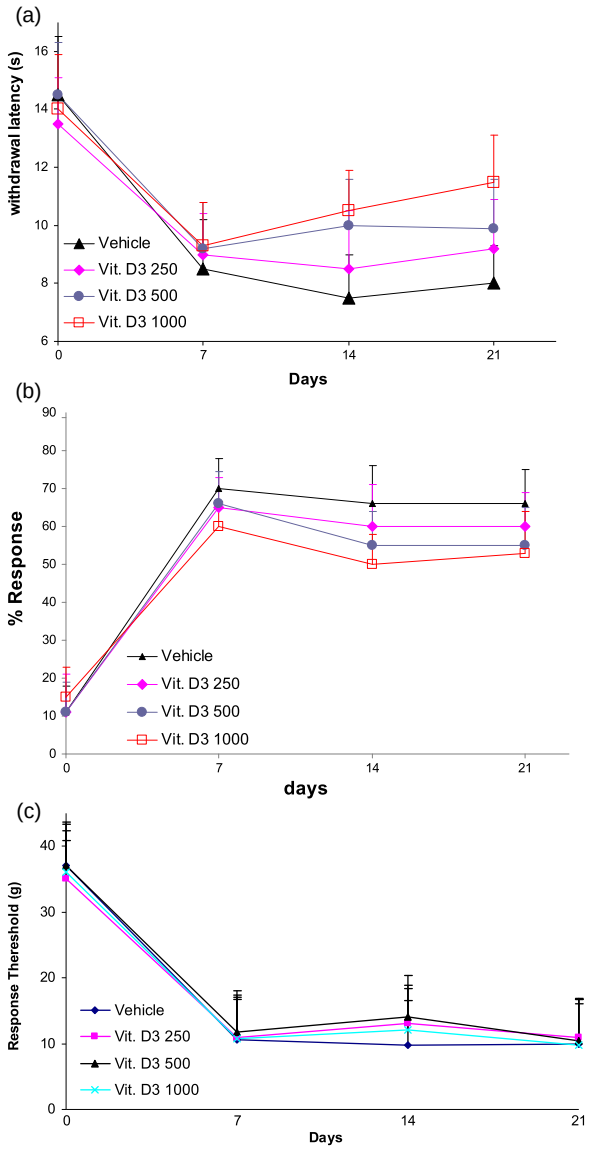

Figure 2 The effects of vitamin D on the expression of neuropathic pain. (A) The effect of acute treatment with vitamin D (250, 500, and 1000 unit/kg i.p.) on the expression of heat hyperalgesia. (B) The effect of acute treatment with vitamin D (250, 500, and 1000 unit/kg i.p.) on the expression of cold allodynia. (C) The effect of acute treatment with vitamin D (250, 500, and 1000 unit/kg i.p.) on the expression of mechanical allodynia. The results are expressed as mean ± S.E.M., $n = 8$ in all groups. Results from von Frey test (Fig. 2C) are expressed as mean rank ± S.E.M. The vehicle groups (CCI) received almond oil according to the treatment schedule.

stimuli. There is a significant enhanced response to the mechanical stimulus in the CCI group compared to the control group ($P < 0.001$). Contralateral hyperalgesia and allodynia did not occur throughout the experiment in all groups.

The effects of vitamin D on the expression of neuropathic pain

Acute administration of vitamin D (250, 500, and 1000 units/kg i.p.) on the 7th, 14th, and 21st days after surgery, 30 min before the behavioral tests did not produce any significant changes in the heat hyperalgesia, cold, and mechanical allodynia in the nerve-ligated rats when compared to the vehicle group (Fig. 2A, B, and C). These results indicate that single administration of vitamin D did not affect the expression of neuropathic pain.

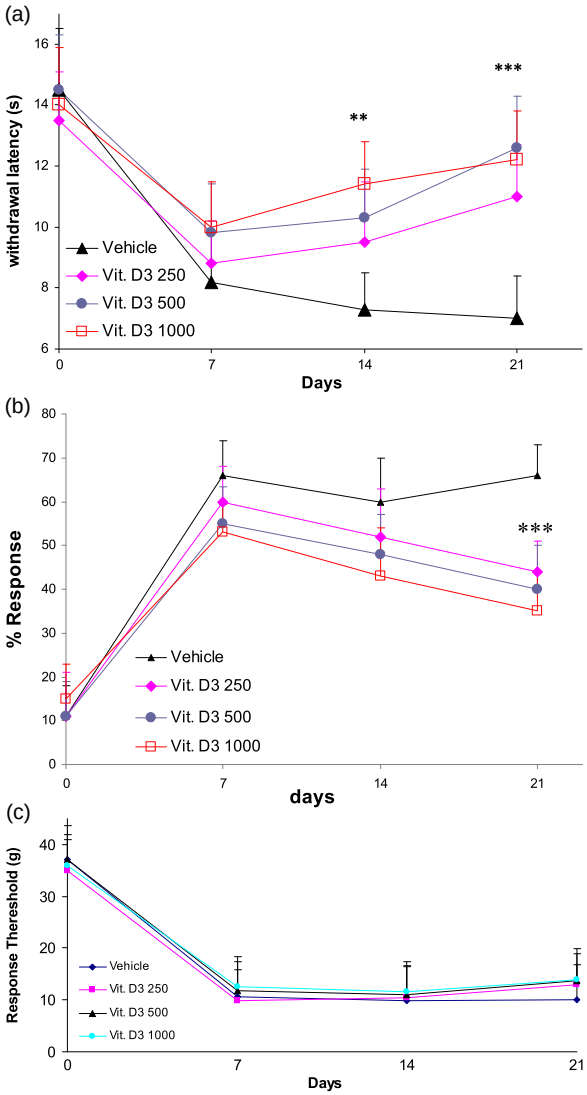

Figure 3 The effects of vitamin D on the development of neuropathic pain. (A) The effect of chronic treatment with vitamin D (250, 500 and 1000 unit/kg i.p.) from 1st day to the 21st day after surgery on the development of heat hyperalgesia. (B) The effect of chronic treatment with vitamin D (250, 500, and 1000 unit/kg i.p.) from 1st day to the 21st day after surgery on the development of cold allodynia. (C) The effect of chronic treatment with vitamin D (250, 500, and 1000 unit/kg i.p.) from 1st day to the 21st day after surgery on the development of mechanical allodynia. The results are expressed as mean ± S.E.M., $n = 8$ in all groups.$P < 0.01$, ***$P < 0.001$ versus vehicle group. Results from von Frey test (Fig. 3C) are expressed as mean rank ± S.E.M. The vehicle groups (CCI) received almond oil according to the treatment schedule.**

The effects of vitamin D on the development of neuropathic pain

To study the effects of vitamin D on the development of neuropathic pain, it was administered repeatedly from the first day after surgery until the 21st post-surgery day. Fig. 3A shows the effects of vitamin D on the development of thermal hyperalgesia. Repeated administration of vitamin D (1000 unit/kg i.p., $P < 0.01$) increased the paw withdrawal latency on the 14th and (500 and 1000 unit/kg i.p., $P < 0.001$) on the 21st post-surgery days. Moreover, chronic administration of vitamin D (250, 500 and 1000 unit/kg i.p.) significantly attenuates cold allodynia (Fig. 3B) ($P < 0.001$). Finally, chronic administration of vitamin D could not significantly modify withdrawal threshold of the nerve-ligated paw (Fig. 3C).

Discussion

Considering our results acute treatment with vitamin D on the 7th, 14th, and 21st days after surgery did not alter pain hypersensitivity compared to the vehicle-treated rats. Although when vitamin D administration was started on the first day after surgery and given daily until the 21st day, cold allodynia and heat hyperalgesia significantly decreased. However, chronic administration of vitamin D could not affect hypersensitivity to the mechanical stimulus in nerve-ligated paw. This difference is probably due to different nociceptors and neuronal pathways involved in different sensory modalities. Vitamin D receptors (VDRs) are widely distributed in the central nervous system.[26,27] Analysis of rat's dorsal root ganglia (DRG) revealed the presence of VDRs and its metabolizing enzymes in the nociceptive neurons of DRG.[28] Vitamin D insufficiency impacts sensory processes such as pain and nociception. Moreover, inflammatory events disturb the VDR signaling in the central nervous system.[29] Also, a significant increase in VDR expression was observed in DRG neurons following neuropathic pain. Furthermore, the greatest increase in VDR expression was observed in small diameter neurons.[30] Consequently, vitamin D may affect sensory neurons especially, small diameter neurons. Considering our results, acute or chronic vitamin D administration did not affect mechanical allodynia. Non-noxious mechanical stimulus is transmitted primarily through the low threshold, large diameter, myelinated A_β fibers, while the cold stimulus

is chiefly transmitted to the spinal cord through the high threshold, thin unmyelinated primary C-fiber nociceptors.[31] Probably due to the greatest expression of VDRs in small diameter neurons following neuropathic pain, vitamin D was ineffective on mechanical allodynia. Thus, these results indicate that vitamin D, when administered immediately after nerve injury, and for a sufficient period of time, can prevent the development of cold allodynia and heat hyperalgesia in rats. This is in accordance with results obtained by Lee et al. Their results showed that vitamin D repletion in diabetic subjects with painful diabetic neuropathy decreases pain scores.[32]

Several experimental models of peripheral neuropathy in rats were developed. In this study, we used CCI of the sciatic nerve as an animal model of neuropathic pain.[33–35] Nerve ligation resulted in high degree of hypersensitivity against the thermal and mechanical stimulus. As described, each treatment protocol affected these parameters to varying degrees. So different mechanisms might be involved in the antinociceptive effects of vitamin D. Vitamin D inhibits the synthesis of nitric oxide synthase (iNOS) in microglia and astrocytes.[36] The increased production of NO is important to maintenance the pain hypersensitivity and contributes to the development of central sensitization and involved in the nociceptive process.[37] Inhibition of iNOS by Vitamin D is a potential mechanism for reducing pain and neuronal damage after injury.[38] Vitamin D can modulate neuronal excitability likes other neuroactive steroids.[39] Neuroactive steroids control the plasticity of the nervous system. This includes spontaneous regular firing, intrinsic excitability, action potential duration, and sensitivity to neurotransmitters.[40–42] Vitamin D as a neuroactive steroid triggers a variety of signal transduction systems. Vitamin D modulates brain neurotransmitters and also upregulates the synthesis of neural growth factor (NGF).[43] In the diabetic neuropathic pain model, vitamin D increased NGF production and prevent neurotrophic deficits.[44] There is some evidence that vitamin D produces a neuroprotective effect by decreasing the effects of glucocorticoids and by modulating neuronal calcium ion homeostasis.[45]

Vitamin D inhibits COX-2 expression and influences prostaglandins by stimulating 15-prostaglandin dehydrogenase (15-PGDH) expression. The enzyme 15-PGDH degrades prostaglandins and inhibits prostaglandin-E2 receptor (PGE2) subtypes.[46] Prostaglandins reduce the firing threshold and mediate neuropathic pain in the spinal cord.[47] Vitamin D affects inflammatory pathways associated with chronic pain.[48,49] Vitamin D suppresses TNF-? and macrophage colony-stimulating factor in astrocytes and microglia. TNF-? strongly associated with both peripheral and central sensitization.[50]

Conclusion

These results indicate that vitamin D supplementation can attenuate the hypersensitivity of the neuropathic pain in rats. These results bring up the beneficial effects of vitamin D in the management of neuropathic pain. However, further pre-clinical and clinical studies are needed to validate this effect and establish the effective doses of vitamin D in human, when prescribing for pain.

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Disclosure statements

Contributors None.

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Conflicts of interest None.

Ethics approval None.

References

1 Jensen TS, Baron R, Haanpää M, Kalso E, Loeser JD, Rice AS, *et al.* A new definition of neuropathic pain. Pain 2011;152: 2204–5.
2 Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol 2010;9:807–19.
3 Finnerup NB, Sindrup SH, Jensen TS. The evidence for pharmacological treatment of neuropathic pain. Pain 2010;150: 573–81.
4 Maier C, Baron R, Tölle TR, Binder A, Birbaumer N, Birklein F, *et al.* Quantitative sensory testing in the German research network on neuropathic pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. Pain 2010;150:439–50.
5 Dworkin RH, O'Connor AB, Audette J, Baron R, Gourlay GK, Haanpää ML, *et al.* Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. Mayo Clin Proc 2010;85:S3–14.
6 Attal N, Cruccu G, Baron R, Haanpää M, Hansson P, Jensen TS, *et al.* European Federation of Neurological Societies. EFNS guidelines on the pharmacological treatment of neuropathic pain. Eur J Neurol 2010;17:1113–e88.
7 Jensen TS, Gottrup H, Sindrup SH, Bach FW. The clinical picture of neuropathic pain. Eur J Pharmacol 2001;429:1–11.
8 Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, *et al.* Molecular mechanisms of vitamin D action. Calcif Tissue Int 2013;92:77–98.
9 Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, *et al.* Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
10 Haque UJ, Bartlett SJ. Relationships among vitamin D, disease activity, pain and disability in rheumatoid arthritis. Clin Exp Rheumatol 2010;28:745–7.
11 Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev 2016;96:365–408.
12 Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008;87: 1080S–6S.
13 Cantorna MT. Vitamin D and its role in immunology: multiple sclerosis, and inflammatory bowel disease. Prog Biophys Mol Biol 2006;92:60–4.

14 Schäfers M, Sorkin L. Effect of cytokines on neuronal excitability. Neurosci Lett 2008;437:188–93.
15 Nicholson B, Verma S. Comorbidities in chronic neuropathic pain. Pain Med 2004;5:S9–27.
16 Gotsman I, Shauer A, Zwas DR, Hellman Y, Keren A, Lotan C, *et al*. Vitamin D deficiency is a predictor of reduced survival in patients with heart failure; vitamin D supplementation improves outcome. Eur J Heart Fail 2012;14:357–66.
17 Hajhashemi V, Banafshe HR, Minaiyan M, Mesdaghinia A, Abed A. Antinociceptive effects of venlafaxine in a rat model of peripheral neuropathy: role of alpha2-adrenergic receptors. Eur J Pharmacol 2014;738:230–6.
18 Banafshe HR, Mesdaghinia A, Arani MN, Ramezani MH, Heydari A, Hamidi GA. Lithium attenuates pain-related behavior in a rat model of neuropathic pain: possible involvement of opioid system. Pharmacol Biochem Behav 2012;100:425–30.
19 Banafshe HR, Hajhashemi V, Minaiyan M, Mesdaghinia A, Abed A. Antinociceptive effects of maprotiline in a rat model of peripheral neuropathic pain: possible involvement of opioid system. Iran J Basic Med Sci 2015;18:752–7.
20 Abed A, Hajhashemi V, Banafshe HR, Minaiyan M, Mesdaghinia A. Venlafaxine attenuates heat hyperalgesia independent of adenosine or opioid system in a rat model of peripheral neuropathy. Iran J Pharm Res 2015;14:843–50.
21 Hamidi GA, Jafari-Sabet M, Abed A, Mesdaghinia A, Mahlooji M, Banafshe HR. Gabapentin enhances anti-nociceptive effects of morphine on heat, cold, and mechanical hyperalgesia in a rat model of neuropathic pain. Iran J Basic Med Sci 2014;17:753–9.
22 Hamidi GA, Ramezani MH, Arani MN, Talaei SA, Mesdaghinia A, Banafshe HR. Ethosuximide reduces allodynia and hyperalgesia and potentiates morphine effects in the chronic constriction injury model of neuropathic pain. Eur J Pharmacol 2012;674:260–4.
23 Verdi J, Jafari-Sabet M, Mokhtari R, Mesdaghinia A, Banafshe HR. The effect of progesterone on expression and development of neuropathic pain in a rat model of peripheral neuropathy. Eur J Pharmacol 2013;699:207–12.
24 Banafshe HR, Hamidi GA, Noureddini M, Mirhashemi SM, Mokhtari R, Shoferpour M. Effect of curcumin on diabetic peripheral neuropathic pain: possible involvement of opioid system. Eur J Pharmacol 2014;723:202–6.
25 Conover WJ, Iman RL. Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat 1981;35:124–9.
26 Prüfer K, Veenstra TD, Jirikowski GF, Kumar R. Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord. J Chem Neuroanat 1999;16:135–45.
27 Veenstra TD, Prüfer K, Koenigsberger C, Brimijoin SW, Grande JP, Kumar R. 1,25-Dihydroxyvitamin d3 receptors in the central nervous system of the rat embryo. Brain Res 1998;804:193–205.
28 Tague SE, Smith PG. Vitamin D receptor and enzyme expression in dorsal root ganglia of adult female rats: modulation by ovarian hormones. J Chem Neuroanat 2011;41:1–12.
29 Smolders J, Moen SM, Damoiseaux J, Huitinga I, Holmøy T. Vitamin D in the healthy and inflamed central nervous system: access and function. J Neurol Sci 2011;311:37–43.
30 Filipović N, Ferhatović L, Marelja I, Puljak L, Grković I. Increased vitamin D receptor expression in dorsal root ganglia neurons of diabetic rats. Neurosci Lett 2013;549:140–5.
31 Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. J Clin Invest 2010;120:3760–72.
32 Lee P, Chen R. Vitamin D as an analgesic for patients with type 2 diabetes and neuropathic pain. Arch Intern Med 2008;168:771–2.
33 Amin B, Noorani R, Marjan Razavi B, Hosseinzadeh H. The effect of ethanolic extract of lippia citriodora on rats with chronic constriction injury of neuropathic pain. Cell J 2018;19:528–36.
34 Carballo-Villalobos AI, González-Trujano ME, Pellicer F, Alvarado-Vásquez N, López-Muñoz FJ. Central and peripheral anti-hyperalgesic effects of diosmin in a neuropathic pain model in rats. Biomed Pharmacother 2018;97:310–20.
35 Saffarpour S, Nasirinezhad F. Functional interaction between N-methyl-D-aspartate receptor and ascorbic acid during neuropathic pain induced by chronic constriction injury of the sciatic nerve. J Basic Clin Physiol Pharmacol 2017;28:601–8.
36 Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB J 2001;15:2579–85.
37 Cury Y, Picolo G, Gutierrez VP, Ferreira SH. Pain and analgesia: the dual effect of nitric oxide in the nociceptive system. Nitric Oxide 2011;25:243–54.
38 Marchand F, Perretti M, McMahon SB. Role of the immune system in chronic pain. Nat Rev Neurosci 2005;6:521–32.
39 Kalueff AV, Tuohimaa P. Neurosteroid hormone vitamin D and its utility in clinical nutrition. Curr Opin Clin Nutr Metab Care 2007;10:12–9.
40 Zakon HH. The effects of steroid hormones on electrical activity of excitable cells. Trends Neurosci 1998;21:202–7.
41 Wu FS, Gibbs TT, Farb DH. Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-*D*-aspartate receptor. Mol Pharmacol 1991;40:333–6.
42 Mensah-Nyagan AG, Meyer L, Schaeffer V, Kibaly C, Patte-Mensah C. Evidence for a key role of steroids in the modulation of pain. Psychoneuroendocrinology 2009;34:S169–77.
43 Neveu I, Naveilhan P, Jehan F, Baudet C, Wion D, De Luca HF, *et al*. 1,25-dihydroxyvitamin d3 regulates the synthesis of nerve growth factor in primary cultures of glial cells. Brain Res Mol Brain Res 1994;24:70–6.
44 Riaz S, Malcangio M, Miller M, Tomlinson DR. A vitamin D3 derivative (CB1093) induces nerve growth factor and prevents neurotrophic deficits in streptozotocin-diabetic rats. Diabetologia 1999;42:1308–1313.
45 Harms LR, Burne THJ, Eyles DW, McGrath JJ. Vitamin D and the brain. Best Prac Res Clin Endocrin Met 2011;25:657–69.
46 Feldman D, Krishnan A, Moreno J, Swami S, Peehl DM, Srinivas S. Vitamin D inhibition of the prostaglandin pathway as therapy for prostate cancer. Nutr Rev 2007;65:S113–5.
47 Richardson JD, Vasko MR. Cellular mechanisms of neurogenic inflammation. J Pharmacol Experi Thera 2002;302:839–45.
48 Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system. Trends Endocrinol Metab 2002;13:100–5.
49 Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Ann Rev Immun 1998;16:137–61.
50 Leung L, Cahill CM. TNF-? and neuropathic pain—a review. J Neuro Inflame 2010;7:27–35.