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ORIGINAL ARTICLE

Investigation of antinociceptive effects of vitamin D and EB1089 in rats

Vitamin D ve EB1089'un antinosiseptif etkilerinin sıçanlarda incelenmesi

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Summary

Objectives: The aim of this study is to investigate the effect of vitamin D on pain threshold in rats. In addition, to examine, whether EB1089, which is a vitamin D receptor agonist, can contribute to this mechanism by increasing the effects of the receptor.

Methods: In the study, 24 male Wistar Albino rats of 3 months, an average of 240–260 g, were used. The animals were randomly divided into three groups, eight animals in each group. Groups; control, vitamin D (10 µg/kg), and EB1089 (10 µg/kg). Tail flick and hot plate tests were used to evaluate the antinociceptive effect. Measurements were taken at 0 min before drug administration and at 30, 60, and 90 min after drug administration and times were recorded in seconds. Serotonin levels were also analyzed by ELISA method in plasma obtained from intracardiac blood samples taken at the end of the experiment.

Results: Vitamin D and EB1089 significantly increased the time to endure pain in the tail flick test compared to the control group ($p<0.05$). In the hot plate test, EB1089 group significantly extended the pain threshold compared to the control group ($p<0.05$), while the vitamin D group did not create a significant difference, although it had a higher latency than the control group ($p>0.05$). There was no significant difference between the groups in terms of serotonin levels ($p>0.05$).

Conclusion: As a result of our study, the administration of vitamin D and EB1089 increased the pain threshold in animals and increased pain resistance.

Keywords: EB1089; pain; serotonin; vitamin D.

Özet

Amaç: Bu çalışmanın amacı, vitamin D'nin sıçanlarda ağrı eşiği üzerine olan etkisini araştırmaktır. Ayrıca, bir vitamin D reseptör agonisti olan EB1089'unda, reseptörün etkilerini artırarak bu mekanizmaya katkı sağlayıp sağlamayacağını incelemektir.

Gereç ve Yöntem: Çalışmada 24 adet, üç aylık ortalama 240-260 gram Wistar Albino erkek sıçan kullanıldı. Hayvanlar rastgele her grupta sekiz hayvan olmak üzere üç gruba ayrıldı. Gruplar; kontrol, vitamin D (10 µg/kg) ve EB1089 (10 µg/kg) olarak belirlendi. Antinosiseptif etkinin değerlendirilmesinde tail flick ve hot plate testleri kullanıldı. Ölçümler ilaç uygulamalarından önce sıfırıncı dakika ve ilaç sonrasını takiben 30, 60 ve 90. dakikalarda alındı ve süreler saniye cinsinden kaydedildi. Deney sonunda alınan intrakardiyak kan örneklerinden elde edilen plazmada, ELISA yöntemiyle serotonin düzeyleri de incelendi.

Bulgular: Vitamin D ve EB1089, tail flick testinde kontrol grubuna göre anlamlı düzeyde ağrıya dayanma süresini artırdı ($p<0,05$). Hot plate testinde ise EB1089 grubu, kontrol grubuna göre anlamlı düzeyde ağrı eşiği süresini uzatırken ($p<0,05$), vitamin D grubu, kontrol grubundan yüksek latense sahip olmasına rağmen anlamlı fark oluşturmadı ($p>0,05$). Serotonin düzeyleri açısından ise gruplar arasında anlamlı bir fark bulunamadı ($p>0,05$).

Sonuç: Çalışmanın sonucunda vitamin D ve EB1089 uygulaması hayvanlarda ağrı eşiğini artırarak ağrıya karşı dayanıklılığı artırdı.

Anahtar sözcükler: EB1089; ağrı; serotonin; vitamin D.

Introduction

Pain is a sensory and emotional personalized experience that is unpleasant to present or potential tissue damage.[1] Pain can be classified in various ways according to factors such as duration, severity, source, and etiology. Acute pain, a form of pain, is a normal physiological response to chemical, thermal, or mechanical stimuli associated with surgery, trauma, and acute illness. It lasts for about 3 months. Pain that lasts longer than this period is classified as chronic pain.[2]

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Neuropathic pain occurs as a result of multiple pathophysiological changes in the central nervous system and peripheral nervous system after nerve damage.[3]

Vitamin D is known as the vitamin primarily responsible for bone metabolism and maintenance of calcium and phosphorus homeostasis in the body.[4] It is a fat-soluble, secosteroid prohormone produced in the skin as a result of contact with sunlight, besides being a vitamin.[5] It has been understood that vitamin D is effective not only in calcium and phosphorus metabolism but also in many tissues by detecting the presence of vitamin D receptor (VDR) (such as pancreas, breast, intestine, lung, gonads, T and B lymphocytes, stomach, skin, brain, and heart). Although the pain pathophysiology is unclear, vitamin D deficiency can lead to pain. In recent years, many studies have been conducted on the relationship between vitamin D and pain, and vitamin D deficiency has been associated with headache, abdominal, knee and back pain, persistent musculoskeletal pain, costochondritis, chest pain, and fibromyalgia.[6–14]

Studies show that vitamin D can play a key role in various cellular activities thought to be protective against the development and modulation of chronic pain. It has been found to act as a neuroactive steroid, interfere with the formation and role of neurotrophins, affect prostaglandin action and inflammatory pathways, and inhibit nitric oxide synthase and helper T-cells. Despite increasing research, there is no firm judgment as to how vitamin D or vitamin D supplementation prevents or cures chronic pain. However, emerging data show that vitamin D/VDR plays a role in pain perception by modulating key pain genes. Besides, the hypothesis that vitamin D probably affects pain signaling pathways can be considered biologically. Because, vitamin D and VDR gene expression have been demonstrated in skin (pain signal transmission), dorsal root ganglia (transmission), spinal cord (conduction/modulation), and brain (pain perception) tissues.[15] VDR expression has also been reported in peripheral and central neurons that play a role in pain perception and processing. It is thought that vitamin D synthesized in the skin interacts with nociceptive neuron nerve endings in the skin and directly perceives harmful pro-inflammatory stimuli[16] and also controls TRPV1 channel activity in T-cells.[17,18] The studies have mainly focused on chronic and neuropathic pain in the literature and there are limited works on acute pain.

In our study, the antinociceptive effect of vitamin D was evaluated in the pain model created with thermal stimulus. In addition, the antinociceptive effect of EB1089, a synthetic vitamin D analog 50–200 times stronger than calcitriol,[19] which mainly affects cancer cells and inhibits cell proliferation and differentiation, was investigated.

Material and Methods

Experimental animals and laboratory conditions

Approval was obtained from Erciyes University Animal Experiments Local Ethics Committee for the study (IACUC-2019 19/003) and the study was carried out in accordance with the principles of the Ethics Committee at Erciyes University Experimental and Clinical Research Center. In the study, 24 male Wistar Albino rats of 3 months in the range of 240–260 gr were used. In the experiments, rats were housed in plastic cages, separated into four animals in each cage. They were accommodated in well ventilated, temperature, and humidity standardized rooms (22±3°C temperature, 62±7% humidity) on a 12 h dark 12 h light cycle. During the experimental process, animals were fed with commercial rat feed and tap water with no any restriction. Experiments were carried out between 10.00 and 14.00 h.

Design of working groups

The animals were randomly divided into three groups of eight animals in each one. The groups are control, vitamin D, and EB1089. 1 ml/kg saline was administered to the control group, 10 µg/kg calstriol to the vitamin D group (Vit D), and 10 µg/kg EB1089 to the EB1089 group. Pilot studies have been conducted to determine these effective doses. 1α, 25-Dihydroxyvitamin D3 (calcitriol) and EB1089 were dissolved in ethanol and administered intraperitoneally (i.p.).

Chemical substances used

1α, 25-Dihydroxyvitamin D3 (Santa Cruz Biotechnology), 1 mg
EB1089 (Santa Cruz Biotechnology), 1 mg
ST/5-HT (Serotonin / 5-Hydroxytryptamine) ELISA Kit (Elabscience®)

Antinociceptive assessment

Tail flick test

This method, known as tail pulling, was first described by D'Amour and Smith in 1941.[20] This test

measures the animal's response to pain induced by thermal stimulus. The tail of the rat was placed in focused light with adjustable intensity from a lamp, and the time between the onset of stimulation and tail pulling was recorded as the tail flick latency. All rats without recording the day before the administration, the Tail-flick devices to make learning exercises (May 0703 TF Tail Flick Commat Unit, Ankara, Türkiye) was placed. The tail pull-up time in seconds was recorded for the measurements of all animals at the 0th min before the drug administration and then at the 30th, 60th, and 90th min after the drug administration. The test cutoff latency was set as 15 s to avoid significant permanent damage to the tails of the experimental animals during the test.[21]

Hot plate test

The hot plate test is an another thermal analgesia test that was used to assess the pain threshold. This test, also known as the hot plate test, was developed by Eddy and Leimbach in 1953.[22] In the experiment, subjects were left on a metal surface heated to 52°C. To keep the subjects around a certain area on the heated surface, a heat-resistant cylinder that does not limit their mobility was used. The time until the animal's hind paw licking or jumping motion after being placed on the surface was accepted as the test latency time. All rats without recording the day before the administration, to make learning exercises to the hot-plate apparatus (May 0603 AHP Hot Plate Analgesic Commat, Ankara, Türkiye) was placed. In order not to cause tissue damage, the cutoff time was determined as 40 s. Pain threshold values of all animals at 0 min before drug administration and 30, 60, and 90 min after drug administration were recorded in seconds.

Measurement of serotonin levels

For serotonin analysis, Serotonin/5-Hydroxytryptamine ELISA Kit (E-EL-0033, 96 T) in accordance with the manufacturer's recommendations and protocol; 50 µL plasma samples were added to the wells and 50 µL of Biotinylated Detection Ab working solution was added to each well and incubated at 37°C for 45 min, the plates were aspirated and washed 3 times. 100 µL HRP (Avidin-HorseradishPeroxidase) conjugate working solution was added and incubated for 30 min at 37°C. Plates were washed by aspirating 5 times. 90 µL substrate reagent was added and incubated at 37°C for 15 min. 50 µL stop solution was added.

Statistical analysis

SPSS 26.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis and measurement data were expressed as mean±standard deviation. One-way analysis of variance was used for comparison between groups. Values with $p<0.05$ were considered statistically significant. The graphics were drawn using the Juptyer Notebook Phyton program.

Results

Evaluation of the effects of vitamin D and EB1089 on pain in the tail flick (TF) test

The effects of vitamin D and VDR agonist EB1089 on pain threshold was compared with the control group (TF: 10.42±2.53, 7.77±2.71, 10.65±3.85), while the vitamin D group significantly increased the pain threshold ($p<0.05$) at the 30th, 60th, and 90th min (TF: 14.02±0.89, 13.56±2.45, 13.80±1.17), in the EB1089 group at the 60th min (TF: 11.60±2.65) significantly increased the pain threshold (Fig. 1).

Evaluation of the effects of vitamin D and EB1089 on pain in the hot plate (HP) test

When the hot plate test was examined statistically, the values of the EB1089 group at 30 and 60 min (HP: 29.33±2.89, 27.40±7.75) compared to the control (HP: 18.20±1.51, 19.85±2.88) significantly increased resistance to pain ($p<0.05$), while the vitamin D group (HP: 25.25±3.84, 23.97±1.85) had a higher latency than the control group in the hot plate test. It has a significant difference ($p>0.05$) (Fig. 2).

Evaluation of the effect of vitamin D and EB1089 on plasma serotonin levels

The averages of plasma serotonin levels were determined. Although the EB1089 group had the highest average among the groups, no statistically significant difference was found when compared ($p>0.05$). Serotonin levels of the groups are shown in Table 1.

Limitations

In the present study, the fact that the vitamin D levels of rats were not known before the study and the serotonin levels could not be examined immunohistochemically on the brain tissue are among the limitations of the study.

Discussion

In our study, prolongation of the duration of the pain threshold occurred in animals treated with vitamin D

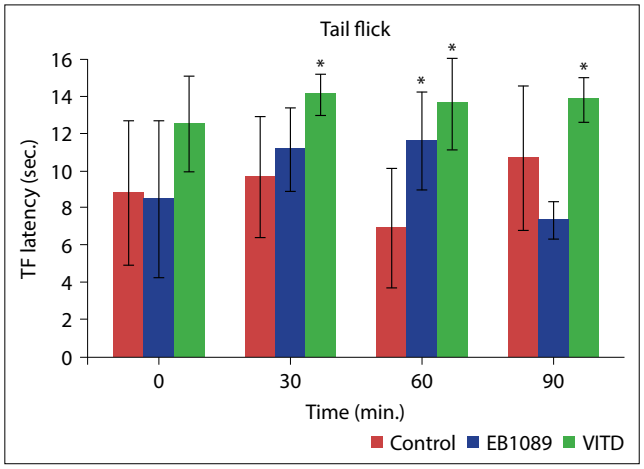

Figure 1. Effects of vitamin D and EB1089 on pain threshold in tail flick test.

*: Significantly different from the control group (p<0.05). VITD: Vitamin D.

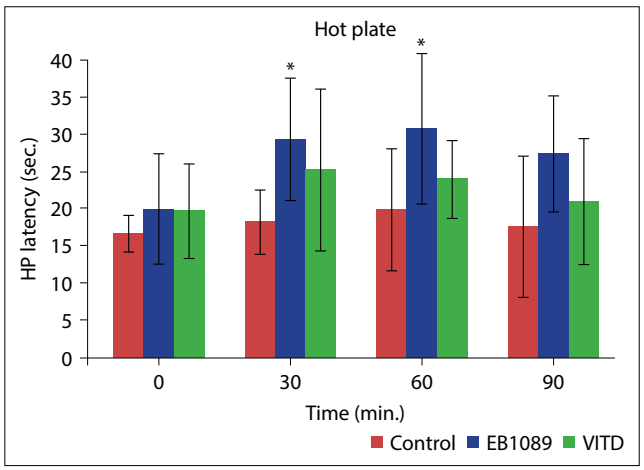

Figure 2. Effects of vitamin D and EB1089 on pain threshold in hot plate test.

*: Significantly different from the control group (p<0.05).

and EB1089. This shows that vitamin D and EB1089 can be effective in the management of pain. When the studies on the relationship between pain and vitamin D are examined, there are studies in the literature with a significant difference and no significant difference, consistent with our study.

No significant difference was found in a study examining chronic musculoskeletal pain and low vitamin D levels in the elderly population.[23] In other studies, low 25-hydroxy vitamin D levels have been associated with a higher incidence of chronic pain.[24,25]

In a study conducted with fibromyalgia patients, 42.6% of the participants had vitamin D deficiency and a significant reduction in symptoms was observed with vitamin D supplementation.[12] Patients with non-specific low back pain received 50,000 IU orally for eight weeks, with no significant difference

Table 1. Serotonin levels

Groups	Serotonin levels (ng/ml)
Control	106.03±63.37
Vitamin D	75.15±50.16
EB1089	117.71±53.88

with the placebo group.[26] In a study examining patients with neuropathic pain due to diabetes, 300,000 IU orally administered vitamin D for 12 weeks has been reported to have a healing effect on pain.[27] Vitamin D replacement also had an ameliorating effect on symptoms in patients with dysmenorrhea.[28] Ergocalciferol, administered orally at 40,000 IU/week for 6 months, reduced the visual pain scale in patients with osteoarthritis.[29] In another study of patients with extensive musculoskeletal pain, ergocalciferol at 50,000 IU/week for 3 months did not have a significant effect on pain.[30]

In rats with a neuropathic pain model, vitamin D administered with a gavage of 1000 IU/kg for 4 weeks significantly reduced pain symptoms.[31] In another study, in rats with sciatic nerve damage, 1000 IU / kg i.p. administered vitamin D reduced behavioral scores of pain.[32]

When the studies are evaluated, the difference in the results varies according to the applied dose, sample size, vitamin D type, and pain models. This situation leads to differentiation in results.

Studies on the effect of vitamin D on pain threshold are limited

In our study, a pain model created with thermal stimulus was used and the relationship between the effect on pain threshold and vitamin D was evaluated. Therefore, calcitriol, which is the most active form with shorter potency and half-life, was preferred. In our study, the group to which we applied vitamin D in the tail flick and hot plate tests created with thermal stimuli to evaluate the pain threshold created a significant level of analgesia compared to the control group.

In this study, EB1089 increased pain tolerance just like calstriol. EB1089 has been found to be able to reduce and inhibit gene expression and enzyme activity in the reference.[33]

In another study, EB1089 has been shown to cause growth arrest and apoptosis in breast cancer cells in culture.[34] EB1089 induced apoptosis activity and produced antiproliferation in hepatocellular cancer cells. This may serve as an example for treatment in clinical practice.[35] Especially in cancer patients, its ability to both reduce pain and slow down tumor growth can provide a two-fold benefit.

In this paper, it was examined whether vitamin D affects serotonin levels. However, no significant difference was found between the groups. Similar to our study, vitamin D supplementation in children with autism alleviated autism symptoms, but did not produce a significant change in serotonin levels.[36] In another study, vitamin D supplementation in children with hyperactivity and attention deficit did not make a significant difference in serotonin levels.[37]

As a result in our work, the increased pain threshold in animals treated with vitamin D and EB1089 showed that these substances can be used in pain management. In addition, EB1089 may be effective in pain management besides its growth-slowing effect on cancerous cells. Thus, it can provide a two-way benefit by increasing resistance to pain, especially in the management of cancer pain. For this, further clinical studies using different pain models are needed.

Ethical Approval: The study was approved by The Erciyes University Animal Experiments Local Ethics Committee (Date: 18/01/2019, No: 19/003).

Conflict-of-interest issues regarding the authorship or article: None declared.

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