Research Article

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Determining the Effect of 1,25 Dihydroxyvitamin D on Pain Threshold in Rats: An Effective Dose Study

1,25 Dihidroksivitamin D'nin Ratlarda Ağrı Eşiği Üzerine Etkisini Belirlemede: Etkin Doz Çalışması

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ABSTRACT

In addition to the known effects of vitamin D on the skeletal system, its effects on other systems have been revealed by studies, and clinical and animal studies have been carried out frequently in recent years. Although there are various forms of vitamin D, the most active and effective is calstriol, known as 1,25(OH)2D. The aim of this study is to determine the effective and safe dose for the evaluation of the effect of calstriol on pain threshold in rats. In the experiment, 32 Wistar Albino male rats, 2.5 months old (240-260 gr) were used. The animals were randomly divided into 4 groups with 8 animals in each group. Groups; control, vitamin D= 1 µg/kg, vitamin D= 10 µg/kg and vitamin D= 100 µg/kg. Tail flick and hot plate tests were used to evaluate the pain threshold. Measurements were taken at the 0th minute before the drug administration and at the 30th, 60th and 90th minutes after the drug administration, and the times were recorded in seconds. Vitamin D= 10 µg/kg administered group significantly prolonged the pain tolerance time in both tail flick and hot plate test compared to the other groups ($p<0.05$). Thanks to the effective dose obtained as a result of our study, experimental pain models using calstriol will be facilitated and further studies will be contributed without wasting extra time and animal loss for the researchers.

Keywords: Calcitriol, pain, tail flick, hot plate, vitamin D

ÖZET

Vitamin D'nin iskelet sisteminde bilinen etkileri dışında diğer sistemler üzerinde de etkisi yapılan çalışmalarla ortaya çıkarılmış olup, son yıllarda klinik ve hayvan çalışmaları sıklıkla yapılmaktadır. D vitaminin çeşitli formları bulunmakla beraber en aktif ve etkili olanı, $1,25(OH)_2D$ olarak bilinen kalstrioldur. Bu çalışmanın amacı ratlarda kalstriolun ağrı eşiği üzerine etkisinin değerlendirmesinde etkin ve güvenilir dozu saptamaktır. Deneyde 32 adet, Wistar Albino cinsi 2,5 aylık (240-260 gr) erkek sıçan kullanıldı. Hayvanlar her grupta 8 hayvan olacak şekilde rastgele 4 gruba ayrıldı. Gruplar; kontrol, vitamin D= 1 µg/kg, vitamin D= 10 µg/kg ve vitamin D= 100 µg/kg olarak belirlendi. Ağrı eşiğinin değerlendirilmesinde tail flick ve hot plate testleri kullanıldı. Ölçümler ilaç uygulamalarından önce 0. dakika ve ilaç sonrasını takiben 30, 60 ve 90. dakikalarda alındı ve süreler saniye cinsinden kaydedildi. Vitamin D= 10 µg/kg uygulanan grup diğer gruplara göre hem tail flick hem de hot plate testinde ağrıya

dayanma süresini anlamlı olarak uzattı ($p<0.05$). Çalışmamız sonucunda elde edilen etkin doz sayesinde, kalstriol kullanılarak yapılacak deneysel ağrı modellerinde kolaylık sağlanacak ve çalışmacılar için ekstra zaman kaybı ve hayvan kaybı yaşanmadan ileri çalışmalara katkı sağlanmış olacaktır.

Anahtar kelimeler: Kalsitriol, ağrı, hot plate, tail flick, vitamin D

1. Introduction

Vitamin D is a fat-soluble, secosteroid prohormone produced from 7-dehydrocholesterol in the skin as a result of contact with sunlight. This produced substance is a precursor, and it is transformed into a biologically active substance by being transformed twice in the liver and kidney [1]. Vitamin D produced in the skin and taken externally, cytochrome p450 and 25-hydroxylase in the liver. It is converted to 25(OH)D by the enzyme. Calcidiol is hydroxylated at position 1 in the kidney with the enzyme 1α hydroxylase, which turns into 1,25 hydroxycholecalciferol or 1,25 hydroxyergocalciferol (1,25(OH)D2) and the resulting substance is called 1,25(OH)2D "calcitriol" [2]. The most active form, calcitriol, is much stronger than 25(OH)D in terms of effect and its half-life is approximately 4-15 hours [1].

The active metabolite of vitamin D, 1,25(OH)2D, exerts its effects through vitamin D receptors (VDR) located in the cytoplasm and nucleus of target cells. Vitamin D receptors are also found in skin, breast, pituitary, parathyroid gland, pancreatic beta cells, gonads, brain, skeletal muscle, circulating monocytes, and activated T and B lymphocytes [3]. Although the physiological functions of these cells are still not fully understood, the number of clinical and experimental studies on vitamin D has increased recently . Although the pathophysiology of pain 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 [4-12]. In this study, the active form of calcitrol was carried out to determine the effective and safe dose on the pain threshold in experimental pain models.

2. Material and Methods

2.1. Experimental Animals and Laboratory Conditions

The study was approved by the Erciyes University Animal Experiments Local Ethics Committee (No: 19/003) and the study was carried out in Erciyes University Experimental and Clinical Research Center in accordance with the principles of the Ethics Committee. In the study, 32 Wistar Albino male rats, 3 months old and 240-260 gr, were used. In the experiments, rats were housed in plastic cages, separated by four animals in each cage, in well-ventilated, temperature and humidity standardized (22 ± 3 °C temperature, 62% ± 7% humidity) rooms in a 12-hour dark and 12-hour light cycle. During the experiment, the animals were fed with commercial rat chow and tap water, and no food or water restriction was applied to the animals during the experiment. Experiments were carried out between 10.00-14.00.

2.2. Design of Working Groups

Animals were randomly divided into 3 groups with 8 animals in each group. Groups; control, vitamin D= 1 µg/kg, vitamin D= 10 µg/kg and vitamin D = 100 µg/kg. 1 ml/kg of physiological saline was administered to the control group. In our study, 1a,25 Dihydroxyvitamin D3, which was applied to the experimental groups, was dissolved using 1 mg (Santa cruz, sc-202877A) ethanol and administered intraperitoneally (i.p.). While planning at the beginning of the study, a sham group was not formed for ethanol, as we benefited from the thesis study that showed that these substances did not have any effect in the tail flick and hot plate measurements made by giving ethanol in the scanned literature [13].

2.3. Evaluating Pain Threshold

2.3.1. Tail Flick Test

This method, known as tail pulling, was first described by D'Amour and Smith in 1941 [14]. This test measures the animal's response to thermal stimulus-induced pain. The tail of the rat was placed in focused light from a lamp with adjustable intensity, and the time between the start of stimulation and tail pulling was recorded as the tail flick Latency = TFL. One day before drug administration, all rats were placed on a tail-flick device (May TF 0703 Tail Flick Unit Commat, Ankara, Turkey) to practice learning. The measurements of all animals at 0 minutes be-

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fore drug administration and then at 30, 60 and 90 minutes after drug administration were recorded in seconds. During the test, the cut-off latency was determined as 15 seconds in order to avoid significant and permanent damage to the tails of the experimental animals [15].

2.3.2. Hot Plate Test

Another thermal analgesia test, the hot plate test, was used to evaluate the pain threshold. This test, also known as the hot plate test, was developed by Eddy and Leimbach in 1953 [16]. In the experiment, the subjects were left on the metal surface heated up to 52ºC. In order for the subjects to stay around a certain area on the heated surface, a heat-resistant cylinder that would not limit their mobility was used. The test latency period was considered as the time from the time to the animal's hind leg licking or jumping after being released to the surface. One day before drug administration, all rats were placed on a hot-plate device (May AHP 0603 Analgesic Hot Plate Commat, Ankara, Turkey) to practice learning, without registration. In order not to cause tissue damage, the cut-off time was determined as 40 seconds. Pain threshold values were recorded in seconds for all animals before drug administration at 0 minute and then at 30, 60 and 90 minutes after drug administration.

2.4. Statistical Analysis

SPSS 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) program was used for statistical analysis and measurement data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used for intergroup comparison. Values with $p<0.05$ were considered statistically significant.

3. Results

In order to evaluate the effect of 1,25(OH)2D on the pain threshold, the response against to pain was measured at 0, 30, 60 and 90 minutes with tail flick (TF) and hot plate (HP) tests using three different doses (1, 10 and 100 µg). In the experiment, 6 were died in 8 animals within vitamin D= 100 µg/kg group. Therefore, measurements with this group were not be completed. In the tail flick test, vitamin D = 10µg/kg group significantly incrreased pain tolerance ($p<0.05$) according to control group (9,67 ± 1,15, 6,90 ± 1,13) and vitamin D= 1µg group (10,07 ± 0,73, 9,65 ± 0,93) in 30th (TF: 14,02 ± 0,38) and 60th (13,56 ± 0,86) minutes. Figure 1 shows the comparisons of the tail flick test of the groups.

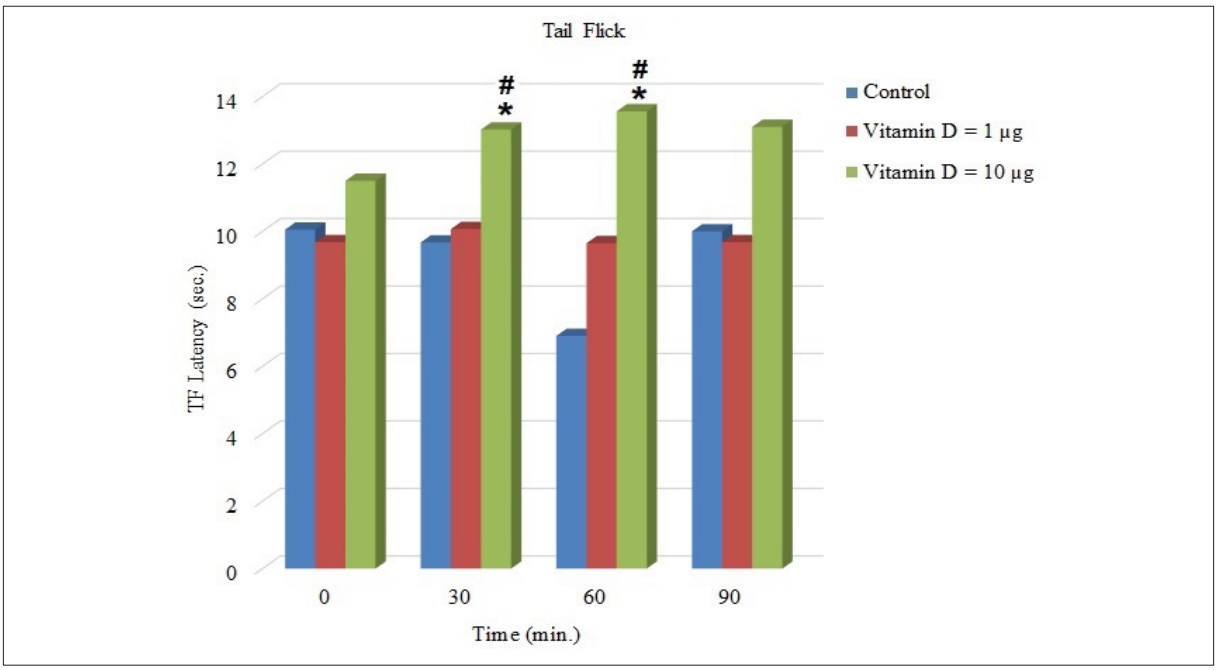

Fig 1. Effects of different doses of vitamin D on pain threshold in tail flick test
* Significantly different from the control group ($p<0.05$)
# Significantly different from the vitamin D= 1 µg/kg

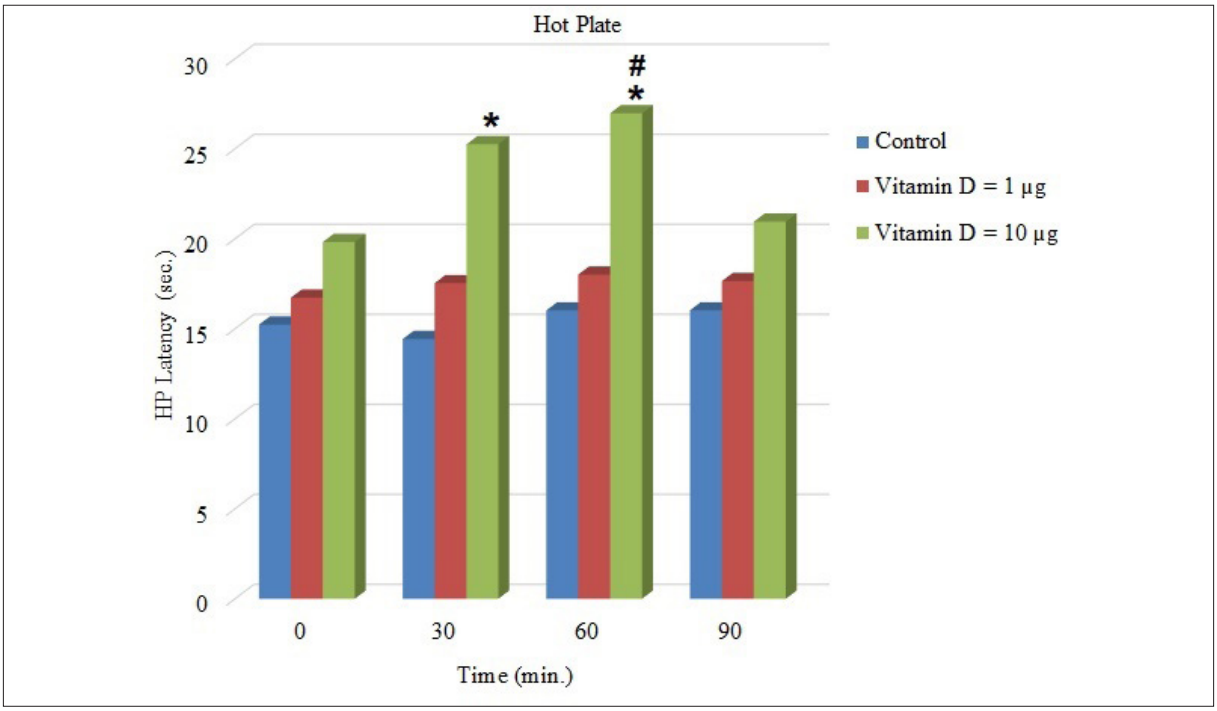

Fig 2. Effects of different doses of vitamin D on pain threshold in hot plate test
* Significantly different from the control group ($p<0.05$)
# Significantly different from the vitamin D= 1 µg/kg

When the hot plate test was analyzed statistically, it is observed that the vitamin D= 10 µg/kg group significantly increased the pain threshold according to control group (HP: 14.43 ± 0.9). at 30th minutes (HP: 25.25 ± 3.84). At the 60th minute, vitamin D= 10 µg/kg group (HP: 23.97 ± 1.85) significantly increased pain tolerance ($p<0.05$) in comparison with control group (HP: 16.02 ± 1.27) and vitamin D=1µg group (18.01 ± 1 ,15). The comparison of groups through tail flick test is shown in Figure 2.

4. Discussion

In clinical studies, a dose of 100 µg/day of vitamin D was found to be an effective and safe dose for adults [17-18]. However, in different animal studies, mostly 1, 5, 10 µg doses of calstriol have been studied in different experiments [19-21] and there are no studies with high doses. For this reason, we wanted to test the effect and safety of this dose by administering 100 µg/kg calcitirol to one of the groups in our study. However, we could not complete the analgesia tests of this group when 6 of the 8 animals in the group died. Animal deaths were thought to be most likely due to excessive exposure to ethanol, the drug's solvent, or another possible high vitamin D toxicity. For this drug in solid form, ethanol, methanol and dimethyl sulfoxide (DMSO) are used as solvents. However, in most of the studies, the solvent of the drug was not written [22-24] or different solvents were used [25-27]. In order to reduce animal deaths that may occur in other studies to be carried out with 1a,25 Dihydroxyvitamin D3 in solid form, it is also recommended to dissolve the drug in less ethanol and to obtain a more concentrated drug, thereby reducing the amount of applied volume.

5. Conclusions

As a result, it has not been presented whether an effective dose study was conducted as a pilot application in many studies. Therefore, studies lead to loss of animals at the planning stage or meaningless results because the effective dose was not known. In this study, the effective dose of calcitriol, which is the active form, was determined by examining the different dose ranges in the literature. Thus, this data can be used for experimental animal studies in similar pain models.

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Limitations

The limitations of our study are the absence of a control group to which we gave ethanol and a more concentrated soluble vitamin D group.

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