International Journal of Research in Medical Sciences
David A et al. Int J Res Med Sci. 2017 Jan;5(1):191-195
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20164547

Original Research Article

An experimental study to evaluate and compare the analgesic activity of calcitriol with morphine in albino mice at a tertiary care teaching hospital in Maharashtra, India

Abhinav David[1], Raj Kumar Goel[1]*, Prashant Patel[2], Rahul Kunkolol[3], Dattatray H. Nandal[3]

[1]Department of Pharmacology, LLRM Medical College, Meerut, Uttar Pradesh, India
[2]Assistant Manager, TCS, Mumbai, Maharashtra, India
[3]Department of Pharmacology, Rural Medical College, Pravara Institute of Medical Sciences, Loni, Ahmednagar, Maharashtra, India

Received: 25 October 2016
Revised: 02 November 2016
Accepted: 29 November 2016

***Correspondence:**
Dr. Raj Kumar Goel,
E-mail: drrajgoel@yahoo.com

ABSTRACT

Background: Vitamin D plays vital role in physiological functions in humans through its active form Calcitriol (Vitamin D3). Vitamin D receptors are found in most tissues, attributing to its classic and non-classic actions. Calcitriol exerts important regulatory effects on the molecular pathways involved in inflammation and pain. The present study is done for experimental evaluation of analgesic action of Calcitriol using pain models in albino mice.
Methods: In this prospective, experimental study, healthy Swiss albino mice were taken after permission from IAEC. Mice were divided into five groups as Control- treated with normal saline, Standard- treated with morphine and Test groups- treated with Calcitriol in dose of 15 µg /kg/mice, 30 µg /kg/mice and 60 µg/kg/mice respectively. Evaluation of analgesic activity was done using Hot plate and Tail flick analgesiometer.
Results: All the 3 test doses of Calcitriol although showed prolongation of reaction time in Hot plate method up to 60 mins but the analgesic activity was not significant in comparison with the standard Morphine. With Tail flick method Calcitriol failed to show any analgesic efficacy at 15-30 µg/Kg but showed some analgesia at 60 µg/Kg which was more than control but not at all comparable with the standard Morphine for thermal pain.
Conclusions: The analgesic activity of Calcitriol was exhibited at higher doses. This property needs to be further evaluated by planning extensive animal experimentation using different animal models.

Keywords: Calcitriol, Escape reaction, Hot plate, Non classic action, Paw withdrawal, Tail flick analgesiometer

INTRODUCTION

Calcitriol also called as Cholecalciferol or Vitamin D3 is one of the major physiologically active forms of Vitamin D. The major endogenous source of Vitamin D for humans is the epidermis.[1] Vitamin D plays a vital role in maintaining serum calcium in a normal range to optimize bone health and other physiological functions throughout the body.[2] In the past few years, there has been growing appreciation for the many roles of Vitamin D and its active metabolites in a large number of tissues. Vitamin D receptors are found in most tissues, attributing to its classic and non-classic actions. Vitamin D receptors located on tissues such as bone, gut, and kidney are responsible for the known classic actions of Calcitriol. However, the discovery of vitamin D receptors in many tissues besides intestine and bone - including brain, heart, pancreas, breast, prostate, lymphocytes, and other tissues

implies that vitamin D supplementation might have applications for treating a number of disorders. These include autoimmune diseases, diabetes, cardiovascular disease, psoriasis, hypoparathyroidism, renal osteodystrophy, and possibly leukemia and cancers of the breast, prostate, or colon.[3-7]

The non-classic actions of Vitamin D can be cell specific and categorized into following general effects: regulation of hormone secretion, regulation of immune function, and regulation of cellular proliferation and differentiation.[8] Calcitriol exerts important regulatory effects on some of the key molecular pathways involved in inflammation, such as inhibition of Prostaglandin synthesis and actions.[9]

The products from the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism are also involved in algesia i.e. pain.[10] All the drugs in clinical practice for pain of inflammation (NSAIDs) act by the mechanism which coincides with Vit D3 action on PGs. The mechanism reveals that Calcitriol can inhibit PG synthesis which is the most important mediator of pain by inhibition of COX-2, down regulation of PG receptors and facilitates inactivation of PGs. However, there are no studies evaluating the acute analgesic effects of Calcitriol.

Experimental evaluation of any drug for the analgesic effects is most commonly carried out using various animal models for nociception. The analgesic activity of a drug for thermal pain is tested using Hot Plate and Tail-Flick Analgesiometer in which time of paw licking and tail flick responses are noted respectively. These responses are measured and recorded before and after the drug administration and can be compared with the standard. Keeping in mind the effect of Calcitriol on the mediators of acute pain it was thought prudent to carry out an experimental evaluation of analgesic action of Calcitriol using above animal pain models in male albino mice.

METHODS

Collection of animals

In the present prospective, experimental study, healthy swiss albino mice of either sex weighing 25-30 gms and age 12 weeks were taken.

The mice were procured from Animal resource centre for medical research, Rural Medical College, Pravara Institute of Medical Sciences, Loni. This study was performed as per the CPCSEA (Committee for the purpose of control and supervision on experiments on animals) after obtaining approval by Institutional Animal Ethics Committee.

All animals were maintained under standard laboratory conditions of food and water before start of the experiment. The animals were housed individually in polypropylene cages containing sterile paddy husk as bedding throughout the experiment. They were given standard pellet diet and water ad libitum throughout the course of study. These mice were maintained under standard condition at temperature of 25±10C, 60±5% relative humidity and 12 hours light dark cycle.

All experiments were carried out between 0900 and 1700 hours according to the guidelines for the care of laboratory animals. Animals were also kept under observation for 7 days, after the completion of experiment to observe any acute or subacute toxicity. All the necessary precautions as per GLP were taken before handling the animals. The mice were held by holding the base of tail with one hand and with the other grasping the loose skin behind the neck.

Drugs and reagents

Injection Morphine sulphate (10 mg/ml) - MORPHITROY*10 (Troikaa Pharmaceuticals Ltd.), Inj. Calcitriol [6, 00, 000 I.U. (15 mg)] - Bone-D3 injection (KEEN Health care (P) Ltd.) and Normal saline- used as a vehicle / solvent were procured for the study. The doses of drugs used were extrapolated from their human equivalent dose (HED) from the values based on data from FDA drafted guidelines for dose conversion based on body surface area.[11]

For the assessment of analgesic activity, three dose levels of test drug Calcitriol were chosen in which the lowest dose is the 50% of the maximum tolerated adult human dose and highest dose is double of the maximum tolerated adult human dose.[12] The drugs were injected through intra-peritoneal route.

Grouping of animals

After one week of acclimatization, the mice were randomly divided into five groups, A,B,C,D and E according to the intervention planned by block randomization method (six in each group). Thus 30 animals were allocated for each experimental model. There are two experimental models thus total 60 mice were taken.

Group A- Control group: Treated with normal saline Dose: 10 ml/kg/mice

Group B- Positive control group: Treated with morphine Dose: 1 mg/kg/mice

Group C- Test group I: Treated with injection Calcitriol Dose: 15 µg /kg/mice

Group D- Test group II: Treated with injection Calcitriol Dose: 30 µg /kg/mice

Group E- Test group III: Treated with Injection Calcitriol Dose: 60 µg/kg/mice

Evaluation of analgesic activity

Hot plate method

The animals were divided into five groups of six animals each. Group A served as control. Group B served as positive control group and were injected morphine sulphate (1 mg/kg, ip). Group C, D and E were treated with injection Calcitriol in dose of 15, 30 and 60 µg /kg/mice, respectively. The animals were individually placed on the hot plate maintained at 55-56°C. The response time (in seconds) was noted as the time at which animals reacted to the pain stimulus by escape reaction (paw withdrawal). The basal reaction time taken by observing hind paw licking or jumping response (whichever appear first) in the animal. The reaction time was calculated before and 30, 60, 120 and 240 minutes after administration of the drug. The reaction time is recorded by a stop watch. A cut-off period of 15 sec was observed to avoid damage to the paws.[13,14] Prolongation of the reaction time in the drug treated animals compared to the control indicates analgesic effect. The percentage increase or decrease in reaction time (as index of analgesia) at each time was calculated.[15]

Percentage increase in reaction time = (Rt/Rc) - 1 x 100

Where, Rt = reaction time in treated group; Rc = reaction time in control group

Tail flick test

The animals were divided into five groups of six animals each. All the animals were marked properly to avoid mixing in two groups. Group A served as control. Group B served as positive control and were injected morphine sulphate. Group C, D and E were injected with calcitriol 15, 30 and 60 µg/kg body weight respectively. After one hour, the tail is kept on the place made for tail above hot wire of the analgesiometer.

The response time (in seconds) was noted as the sudden withdrawal of the tail. Cut off time of 15 seconds was maintained to prevent damage to the tail. The time required for flicking of the tail, was recorded, to assess response to noxious stimulus. The animals were submitted to the same testing procedure before and 30, 60, 120 & 240 minutes after administration of the drug. For each individual animal the reaction time of tail flick was measured and recorded with the help of a stop watch.[13,14] Prolongation of the reaction time (Tail-flick latency) in the drug treated animals compared to the control indicates analgesic effect. The percentage increase or decrease in reaction time (as index of analgesia) at each time was calculated.[15]

Percentage increase in reaction time = (Rt /Rc) - 1 x 100

Where, Rt = reaction time in treated group; Rc = reaction time in control group

Statistical analysis

All values were expresses as mean±SD and compared with the corresponding control values. P- Values are calculated by using one – way ANOVA followed by Tukey – Kramer multiple comparison test. P <0.05 was regarded as statistically significant.

RESULTS

For Hot Plate method, it was found that there was significant prolongation of response time at 30-240 minutes as compared to the response time before giving the drug in group B while C, D, E showed at only 30 and 60 minutes.

Table 1: Comparison of mean and SD values of response (in seconds) by hot plate method in all groups under study.

Group	Drugs	Response Mean±SD (in seconds)				
		Before	After			
			30 min	60 min	120 min	240 min
A	Normal saline (10ml/Kg)	3.5±0.06	3.54±0.05	3.54 ± 0.05	3.53±0.05	3.48±0.05
B	Morphine (1mg/Kg)	3.91±0.06	11.33±0.90 * (271.48)	12.68 ± 0.84* (314.38)	10.19±0.69* (233.01)	7.87±0.45* (161.46)
C	Calcitriol (15µ/Kg)	3.51±0.03	3.36±0.21* (10.16)	3.24±0.20* (5.88)	3.12±0.19 (1.96)	3.06±0.14 (1.66)
D	Calcitriol (30µ /Kg)	3.62±0.07	4.06±0.30* (33.11)	3.77±0.21* (23.20)	3.64±0.22	3.53±0.23 (17.28)
E	Calcitriol (60µ / Kg)	3.72±0.13	4.20±0.14* (37.70)	3.96±0.24* (29.41)	3.74±0.22	3.71±0.25 (23.26)

(Numbers in parenthesis indicate percentage increase in reaction time when compared with control. *p<0.01 when compared with control)

On comparison of mean and SD values of Response (In Seconds) in Hot plate method there was highly significant difference in favor of group B showing highest analgesic activity followed by group E, group D, group C and

group A when compared with each other (i.e. p<0.01). Thus group C, D, E showed analgesia not comparable with the standard group B at 30, 60, 120 and 240 minutes. All the 3 test doses of calcitriol although showed prolongation of reaction time up to 60 minutes but the analgesic activity was not significant in comparison with the standard Morphine (Table 1). By using hot plate method the change in reaction time with the test dose of 15 µg/Kg was less than the control normal saline revealing no analgesic activity for the dose of 15 µg/Kg but the doses of 30 µg/Kg and 60 µg/Kg were found slightly better in terms of prolonging reaction time than the control normal saline revealing slight analgesic activity but not comparable with the standard Morphine.

The results of Tail Flick method exhibit significant prolongation of response time at 30- 240 minutes in only group B and E. The prolongation of response time was only seen with test dose of 60µg/Kg and not observed with other test doses signifying ineffectiveness of calcitriol at 15 µg/Kg and 30 µg/Kg as analgesic (Table 2).

There was highly significant difference in favour of group B showing highest analgesic activity followed by group E, group A, group D and group C when compared with each other (i.e. p<0.01). Thus group C, D, E showed analgesia not comparable with the standard group B at 30, 60, 120 and 240 minutes.

Table 2: Comparison of mean and SD values of response (in seconds) by tail flick method in all groups under study from before and after drug effect.

Group	Drugs	Tail Flick Response Mean±SD (In seconds)				
		Before	After			
			30 min	60 min	120 min	240 min
A	Normal Saline (10ml/Kg)	4.93±0.27	5.05±0.23	4.96±0.23	4.90±0.23	4.84±0.22
B	Morphine (1mg/Kg)	5.04±0.41	9.99±2.20* (97.82)	11.91±2.69* (140.12)	10.40±1.48* (112.24)	9.95±1.52* (105.58)
C	Calcitriol (15µ/Kg)	4.71±0.22	4.81±0.42 (-4.75)	4.85±0.55 (-2.22)	4.79±0.51 (-2.24)	4.76±0.46 (-1.65)
D	Calcitriol (30µ /Kg)	4.76±0.25	4.88±0.41 (-3.37)	4.86±0.39 (-2.02)	4.84±0.37 (-1.22)	4.80±0.38 (-0.83)
E	Calcitriol (60µ / Kg)	5.19±0.06	5.90±0.30* (16.83)	5.63±0.23* (13.51)	5.41±0.21* (10.41)	5.39±0.21* (11.36)

(Numbers in parenthesis indicate percentage increase in reaction time when compared with control. *p<0.01 when compared with control)

Although test dose of 60µg/Kg (Group E) showed favorable effect but that was not comparable to analgesia with Morphine (Table 2). The analgesic activity of Calcitriol (group E) was statistically significant than group A at 30, 60, 120 and 240 minutes. With Tail flick method Calcitriol failed to show any analgesic efficacy at 15-30 µg/Kg but showed some analgesia at 60 µg/Kg which was more than control but not at all comparable with the standard for thermal type of pains.

DISCUSSION

The present study was initiated with an aim of evaluating and comparing a non-classic action of Calcitriol as an analgesic for acute pain similar to the other nonclassic actions of vitamin D. The same was also demonstrated by Daniel bikle in his review stating the utility of Calcitriol in inflammatory disorders.[8] In this study total 60 Swiss albino mice of approximately equal weight and of either sex were used. The analgesic activity of 3 doses of Calcitriol (Group C, D and E) was compared with standard analgesics Morphine (Group B) while Normal saline served as Control (Group A). All the groups were evaluated by using Hot plate and tail flick analgesiometer as experimental models for nociception.

In present study, the doses of Calcitriol (15-30 µg/Kg) did not reveal any analgesic activity when tested on hot plate and tail flick analgesiometer. The dose of 60 µg/Kg of Calcitriol revealed significant analgesic activity as compared to control when tested on hot plate and tail flick for pain but not comparable with the analgesic activity of standard drug Morphine.

The Role of vitamin D as reported by Stewart B extends beyond the bone and its involvement in chronic pain syndromes is also supported by some studies in the literature suggesting the possible benefits of vitamin D supplementation in musculoskeletal pain.[16] There is not much work been done for analgesic activity of Vitamin D. The literature for the same is scanty hence our study lacks comparative data.

CONCLUSION

It can be concluded that Calcitriol is devoid of any non-classic analgesic activity for acute pain at lower dose but higher doses showed an evidence of analgesic activity in experimental conditions and animals we used. The analgesic activity of Calcitriol exhibited at higher doses need to be further evaluated by planning extensive animal experimentation using different animal models.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Adams JS, Hewison M. Update in Vitamin D. J Clin Endocrin Metab. 2010;95(2):471-8.
2. Holick MF. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. Curr Opin Endocrinol Diabetes. 2002;9:87-98.
3. Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. Cancer. 2002;94(6):1867-75.
4. Holick MF. Vitamin D: A millenium perspective. Journal of Cellular Biochemistry. 2003;88(2):296-307.
5. Holick MF. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ, ed Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism 5th ed Washington, DC: Amer Soc Bone Mineral Rese. 2003;5:129-37
6. Johnson LE. Vitamin D. Merck Manual [online] 2007 Available from: http://www.merck.com/ mmpe/ sec01/ ch004/ ch004k.html? qt=vitamin%20D&alt=sh#t b004_6. Accessed 2/19/08
7. Vasquez A, Manso G, Cannell J. The clinical importance of vitamin D (cholecalciferol): a paradigm shirt with implications for all healthcare providers. Alternative Therapies. 2004;10(5):28-36.
8. Bikle D. Nonclassic Actions of Vitamin D. J Clin Endocrin Metab. 2008;94(1):26-34.
9. Krishnan AV, Feldman D. Mechanisms of the Anti-Cancer and Anti-Inflammatory Actions of Vitamin D. Ann Rev Pharmaco Toxico. 2011;51(1):311-36.
10. Hudspith MJ, Siddall PJ, Munglani R. Physiology of pain. HEMMINGS, HC; HOPKINS, M Foundations of Anesthesia [Internet] 2006. Available from: http://www.philippelefevre.com/ downloads/ basic_sciences_articles/ pain-andanalgesia/ nociception.pdf.
11. Reagan-Shaw S, Nihal M, Ahmad, N. Dose translation from animal to human studies revisited. FASEB. 2007;22:659-61.
12. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press. 2010.
13. Vogel HG. Drug discovery and evaluation: pharmacological assays / (ed.).-- 2nd ed. 2002:694-6.
14. Bikash M, Ajay P. Practical Manual of Experimental and Clinical Pharmacology. Edition 2010:201-2
15. Sopan BP, Kshitij RP, Anil CN, Sudhir A, Ananda PRB. Evaluation of Analgesic activity of Hibiscus tiliaceus Linn Wood. World J Pharma Pharmaceu Scie. 2012;1(1):290-7.
16. Leavitt SB. Vitamin D–A neglected "analgesic"for chronic musculoskeletal pain. Pain Treatment Topics [Internet]. 2008. Available from: http://www.thblack.com/ links/ RSD/ vitamind-report.pdf.

Cite this article as: David A, Goel RK, Patel P, Kunkolol R, Nandal DH. An experimental study to evaluate and compare the analgesic activity of calcitriol with morphine in albino mice at a tertiary care teaching hospital in Maharashtra, India. Int J Res Med Sci 2017;5:191-5.