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PŮVODNÍ PRÁCE

Can Intra-articular 1α, 25-Dihydroxyvitamin D3 Administration Be Therapeutical in Joint Cartilage Damage?

Může intraartikulárně aplikovaný 1α, 25-dihydroxyvitamin D3 působit terapeuticky u poškození kloubní chrupavky?

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ABSTRACT

INTRODUCTION

Vitamin D-deficiency is known to cause nerve conduction impairments, cancer and chronic diseases, as well as the pathogenesis of osteoarthritis. Our goal with this study is to evaluate the cartilage healing by applying intraarticular 1α, 25 (OH) 2D3 at different doses in rats with normal vitamin D levels and metabolism, which we made focal chondral damage model in the knee joint.

MATERIAL AND METHODS

35 male Sprague-Dawley rats aged 20–24 weeks were used in our study. Both knees of rats were cartilage defected surgically on day 0. Joint injections performed at 06:00 am on 0th and 2nd days and after second injection others performed on days 9–16 and 23 following a weekly period.

RESULTS

In the fourth week, hematoxylin eosin staining measurements showed statistically significant difference according to the groups ($p < 0.01$) Metalloproteinase-13 (MMP-13) in histological staining for evaluating cartilage healing and healing levels showed statistically significant differences between the groups at first week and fourth week ($p < 0.05$).

DISCUSSION

Vitamin D, which affects many tissues through its receptors, is believed to be chondroprotective and neuroprotective by decreasing the expression of MMP in cartilage fibroblast, macrophage, lymphocyte through its intracellular receptors. To the best of our knowledge, this is the first study known to be intraarticular use of 1α, 25-dihydroxyvitamin D3. Our study has been found to be safe and successful in terms of weight, systemic PTH and 1α, 25-dihydroxyvitamin D3 levels in rats during treatment as well as better healing of cartilage damage.

Key words: vitamin D3 receptor, articular cartilage, orthopedics, nerve conduction.

INTRODUCTION

Osteoarthritis is a common problem which results with cartilage damage and subsequent degeneration progression (8). Because of subchondral bone tissue to becomes open, it results in pain and joint failure, affects 12.1% of the over 25-year-old population in the United States (4). There are many risk factors such as genetic, biological and mechanical in development of osteoarthritis (9). Vitamin D-deficiency is known to cause cardiovascular problems, cancer and chronic diseases, as well as the pathogenesis of osteoarthritis (2, 6).

Vitamin D is a steroidal hormone synthesized in the body from its precursors received from plant and animal nutrients. It is effective on bone mineral density by calcium and phosphorus regulation (11). Vitamin D, which has receptors in many tissues, is also indispensable for the articular cartilage and its effect on cartilage is proven (11, 15). 1α, 25-dihydroxyvitamin D3 [1α, 25(OH)2D3], one of its active metabolites obtained by 1-hydroxylation of 25-hydroxyvitamin D3 in the kidney, provides bone and mineral homeostasis and its integrity also is an important immunomodulatur on that roles in differentiation and proliferation of T and B lymphocytes (7, 17). In cartilage, it takes part in the synthesis of proteoglycan and collagen via its intracellular receptors (7).

Cell culture studies showes that 1α, 25-dihydroxyvitamin D3 alone does not affect matrix metalloproteinases (MMP), but in an inflammatory state such as rheumatoid arthritis which comes with increasing synovial interleukin-1 (IL-1) it increases the expression of MMP-1, MMP-3 and specially MMP-13 which are major factors

of cartilage damage (16). In animal studies, it has been shown clearly that osteoarthritis had a quick progression in animals with 1α, 25(OH)2D3 deficiency and there was an obvious decrease in the expression of major cartilage degradation metalloproteinases such as MMP-13 in animals receiving 1α, 25(OH) systemically (11). There is not enough literature data yet about the in vivo effect of 1α, 25(OH)2D3 on healthy and damaged cartilage with normal vitamin D metabolism and levels.

Our goal with this study is to evaluate the cartilage healing by applying intraarticular 1α, 25(OH)2D3 at different doses in rats with normal vitamin D levels and metabolism, which we made focal chondral damage model in the knee joint.

MATERIAL AND METHODS

Animal groups

35 male Sprague-Dawley rats aged 20–24 weeks were used in our study. Rats were obtained from the Regenerative and Restorative Medicine Research Center (REMER) of Istanbul Medipol University. All rats were followed for 4 weeks, fed ad libitum in 3-way cages at a room temperature of 25 °C, 12 hours of night/12 hours of day on a circadian rhythm.

Rats were randomly divided into 5 groups each containing 7 rats;

- Intact cartilaginous structure and intraarticular saline applied group *(control group-1),*
- Surgically defected in knee joint cartilage and saline applied group *(control group-2),*
- Intact cartilaginous structure and intraarticular 1α, 25(OH)2D3 (Calcijex 1 mcg/ml Hospira S.p.A Milano, Italy) applied group *(study group-1)*,

Vitamin D doses of the rats are adjusted according to the adults weighing in avarege of 70 kg. For adults doses are 250 IU and 1000 IU, for the rats doses are 1 IU and 4 IU.

- Surgically defected in knee joint cartilage and low dose intraarticular 1α, 25(OH)2D3 applied group *(study group 2),*
- Surgically defected in knee joint cartilage and high dose 4 IU intraarticular 1α, 25(OH)2D3 applied group *(study group-3).*

Both knees of rats were cartilage defected surgically on day 0. Joint injections performed at 06:00 am on 0^{th} and 2^{nd} days and after second injection others performed on days 9–16 and 23 following a weekly period. On day 9, 3 rats from each group (6 knees in each group with a total number of 15 rats) sacrified and immunohistologically evaluated to determine acute healing in the joint cartilage. All remaining (20 rats) sacrificed on thirtieth day and immunohistologically evaluated and the study was terminated.

Blood sample

Rats were sampled by 26-G (gauge) needle from the tail vein at day 0 and day 30. And stored -80 °C for Serum 1α, 25(OH)2D3 and parathyroid hormone (PTH) evaluation.

Surgical modeling and injections

At day 0 rat's knees were shaved after general anesthesia with intraperitoneal 50 mg / kg ketamine HCl (Alfamine) and 10 mg/kg Xylazine (Alfazyne) and reached to knee joint by medial parapatellar incision, a 1 cm incision from Joint anterior midline with a 15 point bisturia. To create a fixed defect, a full-thickness cartilage defect

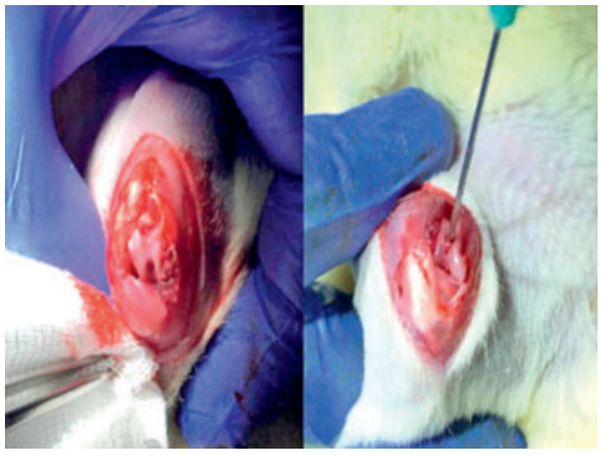

Fig. 1. Surgically creation of cartilage damage in the knee joint.

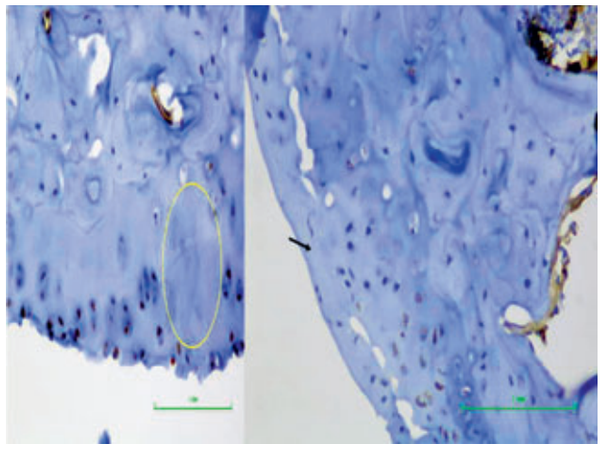

Fig. 2. MMP stained cellulary poor areas (yellow ring), and degenerative area (black arrow).

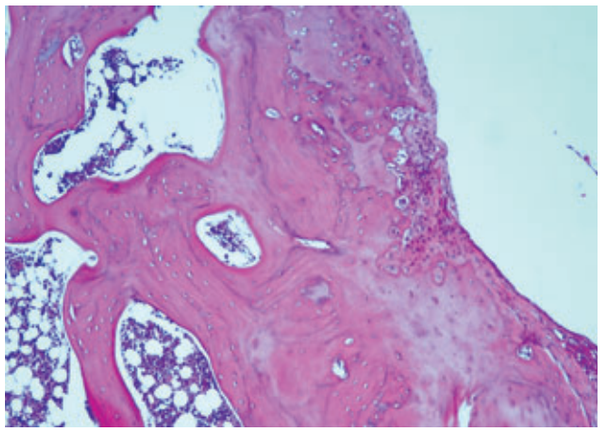

Fig. 3. Healthy cartilage-like formation with H&E staining.

was created at the end of the intercondylars with a 21-G green (Hayat-Siringa) needle (Fig. 1). After clearing the joint with sterile saline, the joint was closed with 4/0 vicryl and the skin closed with 4/0 proline.

Histological evaluation

Tissues fixed with 10% formaldehyde and subsequently held in 10% formic acid, then the articular region sectioned and embedded in paraffin. Sections taken from paraffin blocks were stained with hematoxylin eosin and MMP13 immunostaining and added to positive charged lamellas. Stain MMP 13 (collgenase-3) Ab-1 (clone VIIIA2) mouse monoclonal antibody thermo Scientific, Freemont, CA, USA. Formalin fixed sections were pretreated with citrate prior to immunostaining. The results of staining were based on the count of 100 cells at 40x magnification in the healing cartilage, where if there was no stained cells considered as 0, staining up to 5% was considered as 1(+) and over 5% 2(+). (Fig. 2).

All the staining process done with automatic machines. Animals separated to 3 groups according to the staining characteristics of them for haematoxylin eosin staining. If the cartilage healing was full-fold group-1, the cartilage healing was up to 50% group-2 and if the subchondral bone was exposed and bleeding foci were visible was evaluated as group-3. (Fig. 3).

Statistical evaluation

Statistical analysis was performed by using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program. The Kruskal Wallis test was used for the comparison of the non-normal distribution of the parameters and the Mann-Whitney U test was used for the determination of the group that caused the difference, as well as descriptive statistical methods (mean, standard deviation, median, frequency, ratio). The results were evaluated in a confidence interval of 95% and a significance level of $p < 0.05$.

Table 1. Results of the 1,25 OH Vitamin D3 levels

	N	1,25 OH Vitamin D3 (ng/ml)					*p*
		Mean	SD	Median	Minimum	Maximum	
Control 1	4	23.077	11.50	18.94	14.60	39.83	***0.589***
Control 2	4	31.090	16.22	24.79	19.63	55.14	
Vitamin D	8	18.937	9.85	19.89	7.98	29.95	

Table 2. Distribution of Hematoxylin Eosin staining measurements according to groups

Hemotoxylin Eosilin Staining		1. week					4. week				
		Control 1	Control 2	Study 1	Study 2	Study 3	Control 1	Control 2	Study 1	Study 2	Study 3
1	**n**	6	3	6	3	5	8	2	8	8	8
	%	100.0%	50.0%	100.0%	50.0%	83.3%	100.0%	25.0%	100.0%	100.0%	100.0%
2	**n**	0	1	0	1	1	0	3	0	0	0
	%	0.0%	16.7%	0.0%	16.7%	16.7%	0.0%	37.5%	0.0%	0.0%	0.0%
3	**n**	0	2	0	2	0	0	3	0	0	0
	%	0.0%	33.3%	0.0%	33.3%	0.0%	0.0%	37.5%	0.0%	0.0%	0.0%
[a]*p*		***0.071***					***0.001*****				
[b]Control 1-2		0.058					0.004**				
[b]Control 1- Study 1		1.000					1.000				
[b]Control 1- Study 2		0.058					1.000				
[b]Control 1- Study 3		0.317					1.000				
[b]Control 2- Study 1		0.058					0.004**				
[b]Control 2- Study 2		1.000					0.004**				
[b]Control 2- Study 3		0.180					0.004**				
[b] Study 1-2		0.058					1.000				
[b] Study 1-3		0.317					1.000				
[b] Study 2-3		0.180					1.000				

Table 3. MMP13 staining measurements according to groups

MMP13 Staining		1. week					4. week				
		Control 1	Control 2	Study 1	Study 2	Study 3	Control 1	Control 2	Study 1	Study 2	Study 3
0	n	0	3	0	4	0	0	3	0	0	0
	%	0.0%	50.0%	0.0%	66.7%	0.0%	0.0%	37.5%	0.0%	0.0%	0.0%
1	n	1	3	1	2	4	2	4	0	3	3
	%	16.7%	50.0%	16.7%	33.3%	66.7%	25.0%	50.0%	0.0%	37.5%	37.5%
2	n	5	0	5	0	2	6	1	8	5	5
	%	83.3%	0.0%	83.3%	0.0%	33.3%	75.0%	12.5%	100.0%	62.5%	62.5%
[a]p		*0.001***					*0.003***				

RESULTS

In our study, 35 male Sprague-Dawley rats aged 20-24 weeks were used. There were no complications during the study also during and after the surgery and injection in any rat. PTH and 1,25(OH)Vitamin D3 were assessed with tail vein blood on Day 0 and Day 30 to evaluate the systemic effect of administered intraarticular vitamin D and all of the PTH values were determined to be normal values such as <0.1 ng/ml. Also There was no significant difference between the levels of vitamin D (Table 1) ($p > 0.05$).

Hematoxylin eosin in (H&E) histological staining for evaluating cartilage healing and healing levels did not show statistically significant differences between the groups at first week ($p > 0.05$). In the fourth week, H&E staining measurements showed statistically significant difference according to the groups ($p < 0.01$).When examined to find out from which group the significance is derived ; especially when all intra-articular D-vitamin treated groups were compared with the group in which the surgical defect was formed and the saline was applied (control group-2) Significantly higher H&E staining and better cartilage healing were found in vitamin-D treated groups ($p = 0.004$; $p < 0.01$) (Table 2). Metalloproteinase-13 (MMP-13) in histological staining for evaluating cartilage healing and healing levels showed statistically significant differences between the groups at first week and fourth week ($p < 0.05$) (Table 3).

DISCUSSION

This study showed that intraarticular administration of 1α,25(OH)2D3 has a direct positive effect on cartilage healing in surgically knee joint cartilage defected rats. We found that intraarticular 1α, 25(OH)2D3 increases the cartilage healing by decreasing the expression of MMP-13 in rats which have a normal vitamin D levels and intact metabolism of it.

Cartilage damage and osteoarthritis is a chronic process that interferes with social life and daily functioning, brings serious burden to the patient and the country's economy in treatment, and its frequency of occurrence is accelerated with age (13). The progression of cartilage damage that does not heal and spreads damage to the entire joint cartilage is the initiator of this chronic process. The cartilage loss may result with a painfull joint because of the subchondral rich nerve conduction.- Currently, there is no definite pharmacological, biological or surgical method to completely heal osteoarthritis (5, 13). Vitamin D is essential for a healthy body, and its deficiency is known to result in development of many diseases such as cancer, nerve conduction impairments, obesity, immunity diseases, osteoporosis and osteoarthritis (2, 5, 6). Vitamin D, which affects many tissues through its receptors, is believed to be chondroprotective by decreasing the expression of MMP in cartilage fibroblast, macrophage, lymphocyte through its intracellular receptors (12, 14).

Arden et al., the study concluded that 25-hydroxyvitamin D3, an active metabolite of vitamin D, was used as a supportive treatment in randomized placebo-controlled trials, and that 25-hydroxyvitamin D3 orally administered in 3-year follow-ups did not inhibit osteoarthritis progression (1). Li et al., the protective effect of 1α, 25-dihydroxyvitamin D3, an active D vitamin form, on osteoarthritis and cartilage damage has been shown (10). To the best of our knowledge, this is the first study known to be intraarticular use of 1α, 25-dihydroxyvitamin D3. The success of our study has been found to be safe and successful in terms of weight, systemic PTH and 1α, 25-dihydroxyvitamin D3 levels in rats during treatment as well as better healing of cartilage damage.

Exogenous 1α, 25-dihydroxyvitamin D3 acts on cartilage via TGF-β and PgE, which are activated by TNF-alpha stimulation (1, 5, 16). High TGF-β levels decrease the expression of MMP-9 and MMP-13, resulting in the cartilage protective effect of 1α, 25-dihydroxyvitamin D3 (5). Especially in rheumatoid arthritis and associated cartilage damage, lower levels of 1α,

25-dihydroxyvitamin D3 in the damaged cartilage region increases the expression of MMP-13, thereby increasing cartilage damage (3, 16). The effect of this mechanism on intact or isolated cartilage damage is unclear, and our study suggests that cartilage regeneration is accelerated by reducing MMP-13 expression in rats with a healthy levels and mechanism of vitamin D.

The presence of groups with different doses in our study and our control groups, which include both intact cartilage and defective cartilage groups, can be considered as our strengths. Another important feature is the assessment of both acute and chronic efficiency. Lack of evaluation different types of defects and a chronic defect can be considered as the limit of our work.

CONCLUSIONS

As a result, intraarticular vitamin D can be administered as a therapeutic with intent to accelerate regeneration of cartilage in intraarticular pathology. Patient-based studies should be undertaken to support and develop this practice.

Abbreviations
H&E: hematoxylin eosin
MMP-13: metalloproteinase-13
1α, 25(OH)2D3 : 1α, 25-dihydroxyvitamin D3
PTH: parathyroid hormone
ng/ml: nanogram/milliliter

Ethics approval and consent to participate
This study was unanimously approved by the ethics committee of Medipol University Animal Experiments Local Ethics Committee (İMÜ-HADYEK) with the decision number 15 in 10.02.2016.

Availability of data and material
Data sharing not applicable to this article as no data sets were generated or analysed during the current study.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
EK organised the study and writing. HÇ and FÇ carried out the writing. AK organised the surgeries.FÇ and ME carried out the histological tests.MB and EK designed the study.

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