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Research Article

CLINICAL EFFICACY OF LOCALLY INJECTED CALCITRIOL IN ORTHODONTIC TOOTH MOVEMENT

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ABSTRACT

Orthodontic treatment has two major problems: being lengthy and costly procedure. The present study was designed to evaluate the clinical efficacy of locally injected vitamin D_3 (calcitriol) in accelerating orthodontic teeth movement (OTM) and reducing treatment time and cost in humans. The study was performed on 15 Iraqi adult orthodontic patients within the age range 17-28 years, they are randomly allocated into three groups, each of five patients and treated with either 15 pg, 25 pg, or 40 pg/0.2ml calcitriol diluted with 10% dimethylsulfoxide (DMSO). The maxillary arch of every patient was divided into control (right) and experimental (left) sides. In addition to force application, the right canine received 0.2 ml DMSO injections while the left canine received the calcitriol injections. The follow up period for every patient included five visits at one week intervals through which they received two injections three times and evaluated for OTM, GCf collection and radiographic examination. Statistically non-significant differences were reported for OTM between control and experimental sides, and among the three groups. However, on clinical efficacy basis, the dose of 25 pg calcitriol produced about 51% faster rate of experimental canine movement compared to control, while each of the 15 pg and 40 pg doses resulted in about 10% accelerated OTM. Further more, the periapical radiographs showed no any damaging effect of calcitriol to the surrounding tissues. In conclusion, for the first time we reported that locally injected calcitriol, in dose dependent pattern, is clinical and cost effective in humans.

Keywords: Orthodontic, Calcitriol, Local injection, OTM

INTRODUCTION

Application of mechanical forces to teeth causes tooth movement as a result of the biological responses of the periodontal tissues. In orthodontic tooth movement, mechanical stress appears to evoke biochemical and structural responses in a variety of cell types both *in vivo* and *in vitro* [1,2]. Although current clinical systems in orthodontics use mechanical forces to induce bone remodeling, several researchers have suggested that there might be ways to increase cellular activity with agents more potent than mechanical force alone. Considerable scientific interest has been focused on chemical [3,4] or electrical stimuli in combination with mechanical forces for more rapid bone turnover and faster orthodontic tooth movement [5]. One of the most commonly studied agents in animal and clinical models is prostaglandin E [6,7]. Klein and Raisz reported that prostaglandin E1 (PGE_1) and prostaglandin E_2 (PGE_2) stimulated bone resorption, directly acting on osteoclasts, and had effects similar to those of parathyroid hormone. The role of vitamin D in the maintenance of calcium homeostasis in human beings has been well documented [8]. In particular, the active form of vitamin D, 1, 25-dihydroxycholecalciferol, is one of the most potent stimulators of osteoclastic activity known. It is also involved in the formation of osteoclasts from precursor monocytes and may produce these effects at much lower doses than other hormones such as prostaglandins. Collins and Sinclair [9] as well as Kale *et al*. have reported that the local administration of vitamin D increases the rate of tooth movement in cats and rats respectively; they have emphasized that administration of vitamin D results in a good balance between deposition and resorption of bone and well-modulated bone turnover compared to prostaglandin administration [10]. It has been shown to be a potent stimulator of bone resorption by inducing differentiation of osteoclasts from their precursors, as well as increasing activity of existing osteoclasts [11]. *In vitro* studies have shown that, upon administration of 1, 25-DHCC, osteoblast cell cultures demonstrate a two- to fourfold increase in osteoclastic bone resorption compared to controls. The same results are seen when 1, 25-DHCC is added to osteoclasts incubated alone. But upon administration of actinomycin D, a known inhibitor of osteoblast activity, 1,25-DHCC was unable to stimulate osteoclastic resorption [12]. Moreover, *in vivo* studies appearing in the orthodontic literature have shown increased levels of orthodontic tooth movement upon daily PDL injections of 1, 25-DHCC. The amount of increased tooth movement compared to controls has been reported to be as high as 60% in experimental animal models [9,13]. According to literature survey, no data available about using vitamin D in human trials; so, the present study was designed to evaluate the effect of locally injected 1,25-Dihydroxy-cholecalciferol (Vitamin D_3, Calcitriol) in accelerating orthodontic tooth movement in humans.

MATERIALS AND METHODS

Subjects Selection and Study Design

The present open label study was conducted at Baghdad Teaching Hospital- College of Dentistry/Baghdad University, during the period from October 2010 to April 2011. The study protocol was in accordance with the ethics of the clinical research and approved by the committee of graduate studies in the College of Pharmacy/Baghdad University. After getting consent of the Orthodontic Department at Baghdad Teaching Hospital, sample selection was started by examining patients seeking orthodontic treatment at the postgraduate clinic. Selection of subjects has been done according to that they are orthodontic patients within the age range 17-28 years, with class I and II malocclusion cases that require bilateral maxillary 1st premolars extraction and bilateral maxillary canines retraction (distalization). They should not have any history of chronic systemic illness, syndromes, craniofacial deformities; i.e. clinically healthy subjects, no previous orthodontic treatment, and no history of chronic drug intake; they should have vital teeth with healthy periodontium and no root resorption (examined by dental panoramic radiographs). After clinical examination of 48 patients indicated for the sample criteria and full interpretation of the research aims, signed consents were obtained only from 22 patients and/or their parents to participate as volunteers. Of those 22 patients, only 15 patients completed the research requirements.

Instruments, Chemicals and Methods

All subjects received Pre-adjusted Fixed Appliance treatment (Stainless Steel Roth 0.022" System, Dentaurum Co., Germany), and finished the 1st (leveling and alignment) phase. Maxillary Canine retraction started in the 2nd phase using stainless steel round 0.018" base archwire with a distalizing force of 150 g measured by a pressure gauge [14]. The anchorage for canine retraction included stoppers mesial to 1st molars, 3 orders bends, ligation of 2nd

premolar and 1st molar, and transpalatal bar. All subjects also have been given orthodontic tooth brushes and chlorhexidine mouth wash to maintain good oral hygiene and prevent gingivitis; they have been instructed to take paracetamol only, if an analgesic treatment was required, for the relief of orthodontic treatment pain. The subjects were randomly allocated into three groups, each treated with specific dose of vitamin D_3 (Calcitriol, Mibe, Germany) (15pg, 25pg and 40pg, respectively). For the three groups of patients, the dose of calcitriol has been diluted with 0.2 ml dimethylsulfoxide (DMSO, Bisolve B.V., Netherlands), which has been used as a vehicle. For every subject, the maxillary arch has been divided into experimental (left canine) side and control (right canine) side as previously reported [15]. In the experimental side, the specified dose of calcitriol was locally injected, while the control side received 0.2 ml of DMSO only; these injections were repeated three times for every subject (at 1st, 2nd, and 3rd visits). For both sides, PDL injections have been given locally into the distal side of canines.

Measurement of the Rate of Canine Movement and Gingival Fluid Collection

At each of the 1st three visits, before applying the force for retraction (distalization) of the canines, the distance between the canine and second premolar (at the widest contact areas) was measured (for the control C and experimental E sides) using a digital vernier to the nearest 0.01 mm. After that, injections and force applications were done. At the first visit after deciding the target dose to each patient, the gingival fluid volume was measured before and 1-1.5 hr after the 1st injection. The same is done at the 2nd and 3rd visits. Also it was measured after 7 days of the third injection. The fluid was carefully collected from the distal gingival sulci of maxillary right and left canines, while the surrounding gingival tissues were isolated with cotton rolls and dried carefully with a gentle blast of air directed in an occlusal direction; this was done until there was no evidence of wetness about the gingival margin. A paper point (size 30) was carefully guided into the distal sulcus depth until a slight resistance was felt, the paper point was left in place for 30 seconds and then was immediately stained with 2% alcoholic ninhydrin solution (for 5 seconds) and left to dry. Quantification of the GCF volume was determined by measuring the stained length of the paper point using a digital vernier to the nearest 0.01mm [16].

Radiographic Evaluation

Peri-apical x-rays were performed for the right and left maxillary canines of each patient at the 5th visit (i.e. 30 days after the first injection) to compare the peri-apical and peri-radicular areas at the control and experimental sides [4].

Evaluation of Time and Cost Effectiveness

The greatest influence of calcitriol on E side rate of canine movement has been compared with the control side and the normally known canine movement in order to calculate if there is any treatment time reduction effectiveness of calcitriol. Assuming that the canine retraction usually requires on the average 6 mm space closure, then canine retraction will require normally about 24 weeks. Again the greatest influence of calcitriol on E side has been compared with the C side and the normally known canine movement in order to calculate if there is any cost reduction effectiveness of calcitriol.

Statistical Analysis

All data were statistically evaluated using the Statistical Package for Social Sciences (SPSS) computer program. Wilcoxon Signed-Rank test for testing the difference between pre- and post-injection measurements, and between C and E sides within each group. Kruskal-Wallis One-Way Analysis of Variance among the 3 groups. If any significant difference was found by this test, it would be followed by the Mann-Whitney test between each 2 groups. *P* values<0.05 were considered statistically significant.

RESULTS

Tables 1 showed the mean values of the rate of canine movement after 3 weeks interval at both the C and E sites for groups 1, 2, and 3, respectively; although there are no statistical differences between the C and E sites at the end of treatment period, the E site in groups 1 and 3 showed about 10% higher clinical net rate of canine movement compared to the C site. In group 2, the E site demonstrates 51.0% increase in OTM movement compared to C site at the end of treatment; however, this increase does not reach statistical significance. Although the mean values of the net canine movements (per 3 weeks) at the control and experimental sides for the three groups are not statistically different, group 2 showed the highest clinical acceleration in the rate of canine movement compared to the others. Tables 2 showed the GCF volume mean values (in mm) during 4 visits for groups 1, 2, and 3, respectively; although there is an increase in GCF volume 1.0-1.5 hr after injections in all groups, no statistical differences reported for these values compared to the pre-injection period. When the values of GCF in different groups were compared, no significant differences reported in this respect. Irrespective of the calcitriol dose administered, all the periapical radiographs showed almost similar normal sequence of OTM for both the E and C canines; i.e. compression of the PDL at the pressure (distalization) site, while widening of the PDL at the tension (mesial) site (Figures 1, 2 and 3). Table 3 shows the clinical approximate treatment time (in weeks) and cost reduction effectiveness of calcitriol compared to the control side and the normally known OTM rate, assuming an average canine retraction distance of about 6 mm. Concerning the time reduction, calcitriol can reduce the period for canine retraction about 6 weeks (i.e. 2-3 visits) compared to the control side, while it can reduce it about 12 weeks (4-6 visits) in comparison to the normally known OTM rate. Accordingly, if the treatment cost for the normally known canine retraction phase was (**X**) value, then it is reduced to 1/2 **X** at the E side and 3/4 X at the C side. This formula can be practically utilized according to the cost rate of the institution or the private clinic.

Table 1: Effect of locally injected different doses of calcitriol (15pg, 25pg and 40pg) on the rate of canine movement (OTM in mm) after three weeks treatment

Time intervals	OTM (mm) in C side	OTM (mm) in E side	% difference	*P* value
Group 1 (15pg calcitriol)	1.294±0.61	1.424±0.63 [a]	10.4	0.893
Group 2 (25 pg calcitriol)	1.04±0.33	1.57±0.84 [a]	50.9	0.138
Group 3 (40 pg calcitriol)	1.044±0.3	1.146±0.36 [a]	9.7	0.5

Values are presented as mean±SD; number of sides=5 at each occasion; C= control, E= experimental; values with non-identical superscripts among different experimental groups are significantly different.

Table 2: GCF volume before and 1.0-1.5 hr after injection of either different doses of calcitriol (14pg, 25pg and 40pg) or vehicle (DMSO)

Timing of GCF collection in each group	GCF volume (mm)		*P* value
	C side	E side	
Group 1 (before injection)	4.06±1.56 [a]	3.82±1.45 [a]	0.764
Group 1 (1-1.5 hr after injection)	4.98±1.43 [a]	5.30±1.62 [a]	0.788
Group 2 (before injection)	3.58±1.12 [a]	4.14±1.9 [a]	0.51
Group 2 (1-1.5 hr after injection)	4.71±1.48 [a]	6.15±1.44 [a]	0.186
Group 3 (before injection)	3.55±1.19 [a]	4.37±1.77 [a]	0.135
Group 3 (1-1.5 hr after injection)	4.45±1.22 [a]	5.57±1.38 [a]	0.175

Values are presented as mean±SD; number of sides=5 at each occasion; C= control, E= experimental; values with non-identical superscripts among different experimental groups within the same timing are significantly different.

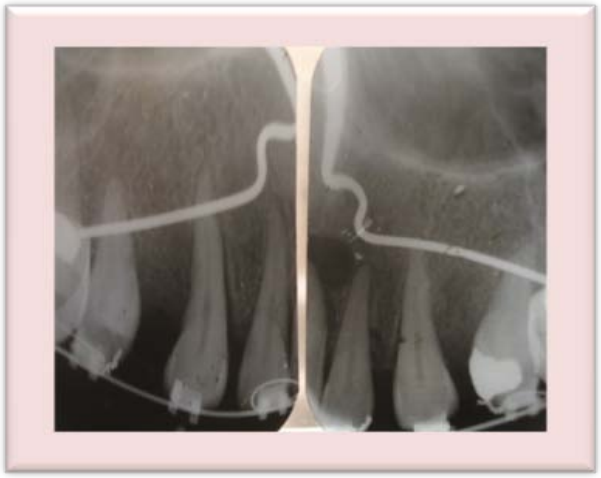

Fig. 1: Right and left peri-apical radiographs for tow patients in group 1

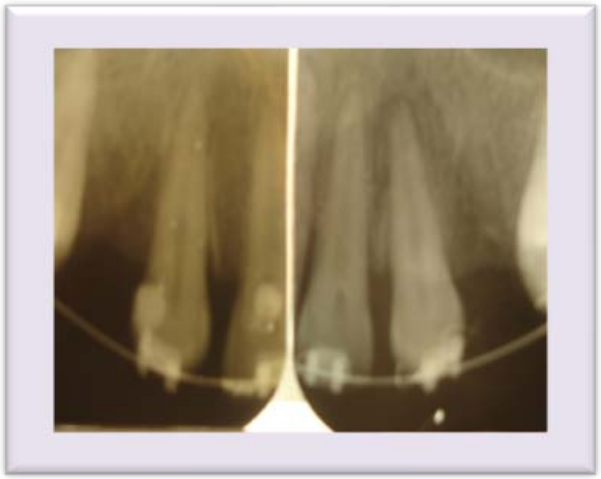

Fig. 2: Right and left periapical radiographs for subject in group 2

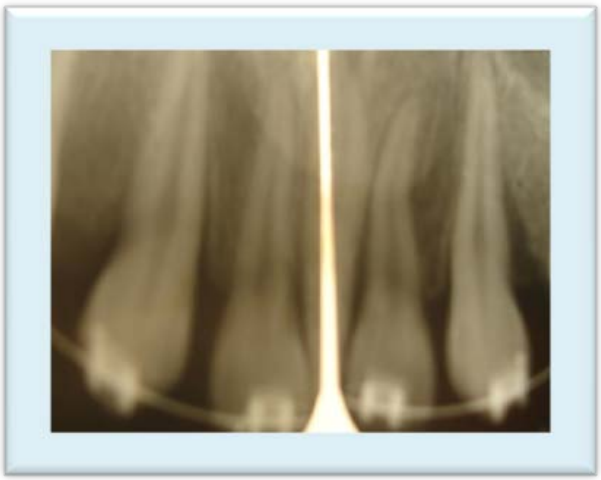

Fig. 3: Right and left periapical radiographs for subject in group 3

Table 3: Approximate estimation of treatment time and cost reduction effectiveness for using locally injected calcitriol in orthodontic procedures

Variable	normal	C	E
Time (weeks)	24	18	12
Cost	X	3X/4	X/2

DISCUSSION

Different methods have been utilized to increase tooth movement and minimize root resorption, such as modifying force magnitude [17], vitamin D metabolite injection [18], steroid therapy [19], altering bone metabolism by PTH [20] and thyroxin intervention. Reports of patients at high risk of developing root resorption suggest the impact of factors other than force [21] and although not definitely proven, a close correlation has been observed between root resorption and hypothyroidism. As previously stated, low levels of serum calcium can also evoke bone and root resorption and a change in serum calcium level is a determining factor for root resorption despite the decisive role of PTH in regulation of bone resorption [22]. It thus seems likely that raised serum calcium levels may inhibit PTH secretion and therefore inhibit root resorption. According to the previously mentioned reports, calcitriol was suggested as a promising choice in orthodontic procedures, and fro the first time we decide to evaluate the clinical utility of such approach; so, the volunteers have been classified into 3 groups that receive different doses of calcitriol (15pg, 25pg, and 40pg). The rational behind choosing these doses is attributed to previous reports about calcitriol, which showed its influence on bone remodeling (resorption and formation) and found to be dose dependent and effective locally within the minimum normal physiological dose [18]. The canine, rather than any other tooth, has been selected because it is the tooth that is most commonly subjected to wide distance movements in clinical orthodontics, making repeated injections and follow up intervals more feasible. Maxillary canine, rather than mandibular canine, has been selected since the maxilla is more frequently included in orthodontic treatment (in comparison to the mandible) with more subjection to canine retractions in so many cases. For the three groups, the comparisons of the rate of canines movement between the C and E sides at each interval were statistically non significant, which is compatible with previously reported data in animal studies [9]; this may be attributed to the small sample size, and the very small values of OTM per a week (parts of a mm). For each of groups 1 and 3, the E side showed about 10% higher clinical net rate of canine movement per three weeks compared to the C side; while this percentage was found to be 51% in group 2 which is comparable to the 60% produced by a calcitriol dose in cats that reported in previous study [9]. This finding comes in agreement with previous animal studies, which indicated that the effect of calcitriol on OTM is highest when administered in doses relatively equivalent to the normal physiologic level [10]. However, the differences among the three groups concerning the net E canine movement per three weeks were statistically non significant, which may be directly attributed to the small sample size; for this reason, the clinical significance and efficacy of calcitriol will be regarded and highlighted, just like so many previous studies in which new techniques, materials, and findings were proved to be clinically efficacious in spite of being statistically non significant [23]. Within each group, both of the C and E sides showed similar patterns of increase or decrease in rate of canine movement per the three intervals, indicating the presence of mild and non significant systemic influence of calcitriol which led the C side to follow the general pattern of the E side; this clinical influence has not been reported or highlighted in previous studies. Moreover, higher rate of OTM in E side was reported compared to the C side at all intervals, this indicates the accelerative action of locally injected calcitriol solution. Meanwhile, the C side in group 2 showed the least net rate of canine movement compared to the other groups, indicating more potent local action of 25 pg calcitriol with minimal systemic influence. It has been demonstrated that calcitriol stimulates bone resorption by inducing the differentiation of osteoclasts from their precursors and increasing the activity of existing osteoclasts in very small doses. In addition, it is more effective than PG in modulating bone turnover during OTM [10]. Quantification of the local inflammatory response to calcitriol has been achieved by GCF collection from the C and E sides before and after each injection. A generally similar behavior in the increase or decrease in GCF volume has been demonstrated by the three groups in relation to timing of GCF collection, where both sides showed generally an increase in GCF volume after each injection with a regression to approximately similar values to those before injection (base line values); such increase in GCF volume is affected by many factors, including presence of gingival inflammation, which is relatively eliminated during the study through adopting proper adherence to good oral hygiene, direct trauma also may play important role especially during PDL injection, and finally the orthodontic movement, where relatively larger GCF volume collected in the E sites which may be attributed to greater acceleration of OTM due to local injection of calcitriol compared to C site. Owing to the back-pressure resistance in the PDL and the confined PDL tissue space, Walton and Abott (1981) and Malamed (1982) recommended that the PDL injection technique must involve slow injection of a relatively small volume (about 0.2ml) of a solution to reduce pain as much as possible [24,25]. That is why this 0.2 ml volume with the finest needle (gauge 30") has been used in this study. Previous animal studies showed that calcitriol could stimulate and increase osteoclastic activity in achieving alveolar bone resorption histologically [10]. It is well known that such an approach is impossible in humans since it requires invasive techniques (e.g. surgical extraction of a tooth with its surrounding alveolar bone). For this reason, Yamasaki *et al*. (1984) used periapical radiographs for assessing whether PG injections have any damaging effects to the surrounding dental tissues [4]; in the present study, the same approach has been followed and the findings were similar to the reported one, indicating that calcitriol has an accelerative not a damaging effect and it is safe when used locally. The assessment of clinical performance is important at the individual, practice, institutional and national levels. It is a challenge not only to deliver high standards of care but also to deliver this care at the lowest units of time and cost [26]. Treatment duration is highly variable from country to another and, to some extent, depends on the type of service in which it is delivered and also the local health care and remuneration system. Treatment duration is also influenced by the severity of malocclusion, training level, experience and aspiration of the clinician, and types of monitoring techniques employed during treatment. An average fixed orthodontic appliance treatment is about 18 months (1.5 year) including about 6 months duration for the 2nd phase (canine retraction or space closure phase) [23]. Previous studies attempted to accelerate OTM and minimize treatment time by calcitriol local administration without any translation of the results into clinical treatment time and cost reduction effectiveness since they were animal studies [9]. In this clinical human study it seems possible to translate the results into clinical benefits of time and cost reduction. Calcitriol administration to the E side was capable of not only reducing treatment time and cost at the E side but at the control side also (but to a lesser extent) as compared to the normally known treatment time and cost (Table 3) since calcitriol appeared to have mild systemic influence. Mandall *et al*. (2006) suggested that 12 weeks reduction in treatment time in comparison with the normally known treatment time is clinically significant in terms of efficiency [23]. In conclusion, local administration of calcitriol, in a dose dependent pattern, is clinical and cost effective in accelerating OTM in humans.

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