TURKISH JOURNAL of ORTHODONTICS

DOI: 10.5152/TurkJOrthod.2020.20014

Original Article

Effect of Strontium Ranelate on Condylar Growth during Mandibular Advancement in Rats

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Cite this article as: Camcı H, Doruk C, Ünver Saraydın S. Effect of Strontium Ranelate on Condylar Growth during Mandibular Advancement in Rats. Turk J Orthod 2020; 33(4): 216-23.

Main points:

- Strontium ranelate (SR), an anti-osteoporotic agent, is composed of one ranelic acid and two non-radioactive strontium atoms. Unlike its counterpart, SR increases bone formation as well as reducing bone destruction.
- Increase in the condylar ossification after mandibular advancement with systemic SR application was evaluated.
- As a result, SR was found to increase condylar ossification at the histological level, but it was determined that SR had no effect on the total size of the mandible.

ABSTRACT

Objective: Strontium ranelate (SR), unlike other anti-osteoporotic agents, might not only prevent bone resorption but also might induce bone formation. The aim of this experimental study was to evaluate the effects of systemic SR on condylar growth during mandibular advancement (MA) in growing rats.

Methods: Fifty-six, 8-week-old Wistar male rats weighting 160-190 g were randomly divided into four groups; one control (n=14) and three experimental (n=14). Group 1: Control group, Group 2: SR (900mg/kg daily dose), Group 3: MA, Group 4: SR +MA. The amount and direction of mandibular growth were assessed by linear measurements on the computed tomography (CT) images taken on days 1, 15, and 30. For immunohistochemical evaluation, half of the subjects in the groups were sacrificed on the 15th day (early phase) and the rest of them on the 30th day (late phase). New cartilage and bone formation areas on the condyle were analyzed by using Sox9 and Osteopontin antibodies.

Results: Early and late CT images measurements showed no significant difference between the groups ($p<0.05$). However, there were significant differences between the control and experimental groups in the immunohistochemical assessment. Severe immunolocalization of SOX9 and Osteopontin was observed in Group 4, while the immunolocalization scores were moderate in Group 2 and Group 3. In addition, early histological findings were similar to late results in all groups.

Conclusion: In mandibular advancement therapy, Strontium ranelate could be therapeutically effective in avoiding relapse and reducing the duration of retention.

Keywords: Mandibular advancement, Strontium ranelate, Computed tomography, Immunohistochemistry

INTRODUCTION

Class II malocclusions are commonly seen in orthodontic practice. This malocclusion might adversely affect facial aesthetics and mastication. Mandibular retrognathia is the primary factor for skeletal Class II malocclusions and a wide range of functional orthopedic appliances are used to correct this anomaly (1). Mandibular retrognathia correction and retention period have an average duration of one year (2). Long treatment periods can weaken patient compliance. In recent studies researchers have tested whether condylar growth can be stimulated during mandibular advancement by different treatment modalities (low-level laser, chemical agents, etc.) (3). These applications were intended both to improve treatment efficiency and to decrease the total treatment duration of mandibular advancement (MA).

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Received: February 14, 2020
Accepted: September 25, 2020
Available Online Date: 27 November 2020

Table 1. Definition of the groups

Groups	Experimental Procedure	Total number of the rats
Group 1(Control)	No application was made.	
	7 rats were sacrificed on the 15th day.	14
	7 rats were sacrificed on the 30th day.	
Group 2 (SR)	Only systemic SR application (900 mg / kg), but no mandibular advancement	
	7 rats were sacrificed on the 15th day.	14
	7 rats were sacrificed on the 30th day.	
Group 3 (MA)	Only mandibular advancement, but no SR application.	
	7 rats were sacrificed on the 15th day.	14
	7 rats were sacrificed on the 30th day.	
Group 4 (SR+MA)	Both mandibular advancement and systemic SR (900 mg / kg) application	
	7 rats were sacrificed on the 15th day.	14
	7 rats were sacrificed on the 30th day.	

SR: Strontium ranelate; MA: Mandibular advancement

One of the major subjects in orthodontics is bone turnover experienced during growth and development periods or orthodontic tooth movements. Anti-osteoporotic agents that affect bone turnover have therefore been the subject of previous orthodontic research (4, 5). Strontium ranelate (SR) is a promising new anti-osteoporotic agent consisting of one ranelic acid and two non-radioactive strontium atoms and has a unique dual-action in bone formation and resorption. It has a good safety profile with tolerability and compliance so it has begun to be used as an alternative to other bisphosphonates for initial osteoporosis treatment. SR is therapeutically indicated for the treatment of severe osteoporosis in both postmenopausal women and adult men at high risk of fractures. Basically, bisphosphonates only prevent the loss of bone density. However, unlike its equivalents, SR increases bone formation, and also reduces bone resorption. SR is the first anti-osteoporotic agent that has dual-effect (6-8). Numerous studies investigating the SR mechanism have shown that it stimulates osteoblast proliferation and inhibits osteoclast formation (9, 10). SR affects bone turnover by stimulating the expression of Osteoprotegerin (OPG), activating the Calcium-sensing receptor (CaSR), and suppressing the Osteoclast differentiation factor (RANKL) (8, 11). Since it enhances osteoblastic activity, previous orthodontic studies have investigated the impact of SR on anchorage and its influence on ossification in the mid-palatal suture (12, 13).

SOX9 is a transcription factor that is expressed in chondrocytes. It is found in both cartilage and primordial cartilage tissues during embryo development and is recognized as the determinant factor for a lineage of chondrocyte (14). Osteopontin regulates the biomineralization of bone tissue. It plays an important role in the osteoblastic activity and is found in bone mineralization regions due to its affinity to calcium (15).

The objective of this experimental study was to investigate the effects of systemic SR on condylar growth during mandibular advancement. In the future, we believe that these kinds of experimental studies might allow clinicians to shorten the retention period of MA treatment and prevent possible relapses.

METHODS

Experimental protocols of this study were approved (17.03.2017-25) by Cumhuriyet University Animal Research Ethics Committee. In the power analysis to determine sample size, it revealed that minimum 14 rats were required for each group in order to obtain sufficient statistical power (n=14, α=0.05, and 1-β=0.80). During the study, all instructions determined by the ethics committee were followed. A total of fifty-six, 8-week-old (160-190 g body weight) Wistar male were used (16, 17). In the selection of experimental animals, attention was paid to conditions such as good general health and ideal anterior teeth. Rats in each group were fed with soft diet and water in a separate cage under the same conditions as 12 hours daily / night, 21±1°C temperature and 40-60% humidity.

Definition of the Groups

Fifty-six growing rats were randomly divided into 4 groups. Half of the animals in each group were sacrificed on the 15th day and the rest of them were sacrificed on the 30th day in order to evaluate the early and late-term effects of SR. The groups were as follows (Table 1):

Group 1 (Control, n=14): This group was included in the study to determine the morphology and normal histological characteristics of condyles and to compare them with experimental groups.

Group 2 (SR, n=14): Daily systemic SR (900 mg/kg) was applied during the experimental period. No mandibular advancement was performed. The group was designed to determine whether or not SR affects condylar growth on its own without MA.

Group 3 (MA, n=14): Mandibular advancement was performed without systemic SR application. This group has allowed us to analyze the nominative effect of the MA on condylar growth.

Group 4 (SR+MA, n=14): First, the placement of mandibular advancement devices was performed, and then the daily systemic SR (900 mg/kg) was given during the experimental period.

Figure 1. Intraoral view of the acrylic bite-jumping appliance

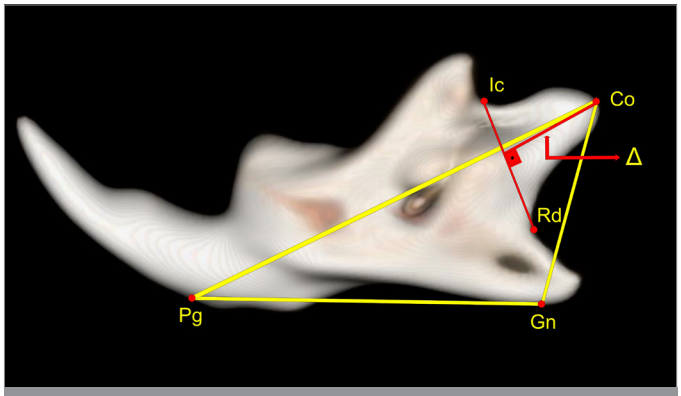

Figure 2. Co: The most posterior-superior point of the condyle, Pg: The lowest point of the mandible near the incisor, Gn: The lowest point of the lower boundary of the angular process, Ic: Deepest point between condyle head and coronoid process, Rd: Deepest point of convexity of mandibular ramus, Co-Gn: Ramus length, Co-Pg: Mandibular length, Pg-Gn: Mandibular base length, Δ: Length of condyle head

Bite-jumping Appliance and SR Application

The animals were anesthetized intraperitoneally to provide immobilization during the appliance placement. After examining the dentoalveolar structure of the subjects, impressions were taken from the lower incisors of the subjects by using silicone impression material. Composite models (Transbond XT, EP7SF, 3 M, Monrovia-CA / US) were produced from the impression and acrylic bite-jumping devices were made to move the lower jaw forward. The appliances were applied to the lower teeth as defined by Owtad et al. (18). Self etch primer (Transbond Plus Self Etching Primer, 359651, 3M, Monrovia-CA / USA) was used to bond the appliances (Figure 1). Through daily checks, the damaged appliances were renewed, and mandibular advancement was obtained again. The food and water intake of subjects were monitored and weight measurements were carried out periodically. The weight of each animal in Group 2 and Group 4 was individually identified and recorded for the preparation of SR (PROTELOS, Servier, France) suspension concentrations. Daily prepared SR (900mg/kg daily dose) suspensions in distilled water were given orally as 1 cc per dose.

Radiological Analysis

The section collimation was set to 0.5 mm and the images were obtained using A Toshiba Aquilino Helical 64-slice tomography device (Canon Group Company, Japan). Computed tomography (CT) images were obtained on the 1st, 15th, and 30th days of the study for the analysis of mandibular dimensional changes. The acquisition of images was performed under intraperitoneal anesthesia in order to prevent motion artifacts. Five reference points were identified and four linear measurements were carried out to evaluate dimensional changes of the condyle and the mandible (Figure 2) (19),(20). Aquarius Intuition Edition Version 4.4 software was used for the analysis.

Histological Evaluations

Immunolocalization is a histological technique in which specific antibodies are used to localize macromolecules (proteins, polysaccharides) within biological material (tissues, cells, biofilms). Before the histological evaluation, the rats were killed by injection of high-dose intraperitoneal anesthesia, and their lower jaws were dissected as a whole. Osteopontin and SOX9 immunolocalizations were assessed to analyze new cartilage and bone formation on the condyle. The following steps were performed during the preparation of the tissues:

- The right condyle heads were fixed in 10% neutral formalin for 30-36 hours
- Decalcification, dehydration, and transparency processes were followed.
- The tissues were embedded in paraffin and 6 µm thick sections were prepared in the sagittal plane.
- Sections were deparaffinized and hydrated in distilled water.
- Anti-SOX9 antibody (Rabbit polyclonal, Bioss) and anti-Osteopontin antibody (Rabbit Mab, Cell Signaling) were used for immunohistochemical staining of the sections. In each section, only the middle part of the condyle was evaluated by a histologist. The selected sections were photographed and digitized. The immunolocalization level of SOX9 and Osteopontin in the new cartilage and bone formation areas were evaluated semi-quantitatively for each subject. The samples were scored as mild (+), moderate (++), and severe (+++).

Statistical Analysis

Statistical analysis were performed by the Statistical Package for Social Sciences, version 15.0 software (SPSS Inc.; Chicago, IL, USA). In the assessment of the CT and histological data non-parametric Kruskal Wallis test was performed to determine the differences between the groups. Mann Whitney U test was also used to determine which group was responsible for the difference. For all statistical comparisons in the study, p values less than 0.05 were considered statistically significant. In addition, Ten randomly selected CT images were re-analyzed 10 days later by the same researcher (H.C.) to assess the error rate. Repeated measurements were compared with the intraclass correlation coefficient (ICCs).

Table 2. Comparison of the body weight measurements (Kruskal-Wallis Test)

	Group 1 (n=14) Mean±SD	Group 2 (n=14) Mean±SD	Group 3 (n=14) Mean±SD	Group 4 (n=14) Mean±SD	p
1st week	160.0±5.4	161.3±7.9	162.0±5.6	161.4±3.5	0.992
2nd week	174.1±7.0	175.0± 12.9	174.1±4.6	173.2±2.9	0.849
3rd week	184.3±7.8	182.0±14.5	184.2±6.0	181.1±4.1	0.936
4th week	194.7±8.0	195.0±12.8	194.6±5.5	193.4±3.8	0.916

SD: Standard deviation, Body weight measurement in gr.

Table 3. Pearson correlation coefficient of the CT measurements

Lengths	Co-Gn	Co-Pg	Pg-Gn	Δ
Correlation coefficient	0.958	0.898	0.937	0.910

Table 4. Comparison of linear CT measurements at T0, T1, T2

1st day (T0)	Group 1 (n=14)	Group 2 (n=14)	Group 3 (n=14)	Group 4 (n=14)	p
Co-Gn	9.1±0.3	9.2±0.2	9.3±0.3	9.2±0.4	0.745
Co-Pg	18.6±0.3	18.8±0.6	18.9±0.4	18.8±0.6	0.669
Pg-Gn	13.4±0.3	13.5±0.7	13.3±0.3	13.2±0.7	0.871
Δ	4.2±0.1	4.2±0.3	4.6±0.3	4.2±0.4	0.590
15th day (T1)	**Group 1 (n=14)**	**Group 2 (n=14)**	**Group 3 (n=14)**	**Group 4 (n=14)**	**p**
Co-Gn	9.6±0.3	9.9±0.4	9.8±0.3	9.8±0.4	0.687
Co-Pg	19.4±0.4	19.9±0.5	20.0±0.5	19.7±0.6	0.506
Pg-Gn	14.1±0.6	14.4±0.5	14.1±0.5	14.0±0.4	0.461
Δ	4.2±0.2	4.3±0.3	4.8±0.3	4.3±0.4	0.550
30th day (T2)	**Group 1 (n=7)**	**Group 2 (n=7)**	**Group 3 (n=7)**	**Group 4 (n=7)**	**p**
Co-Gn	10.2±0.4	10.4 ±0.4	10.5±0.2	10.4±0.4	0.669
Co-Pg	20.5±0.4	20.8±0.6	20.9±0.4	20.8±0.5	0.962
Pg-Gn	14.7±0.6	14.7±0.6	14.3±0.5	14.2±0.5	0.770
Δ	4.3±0.2	4.3±0.3	4.9±0.3	4.6±0.5	0.161

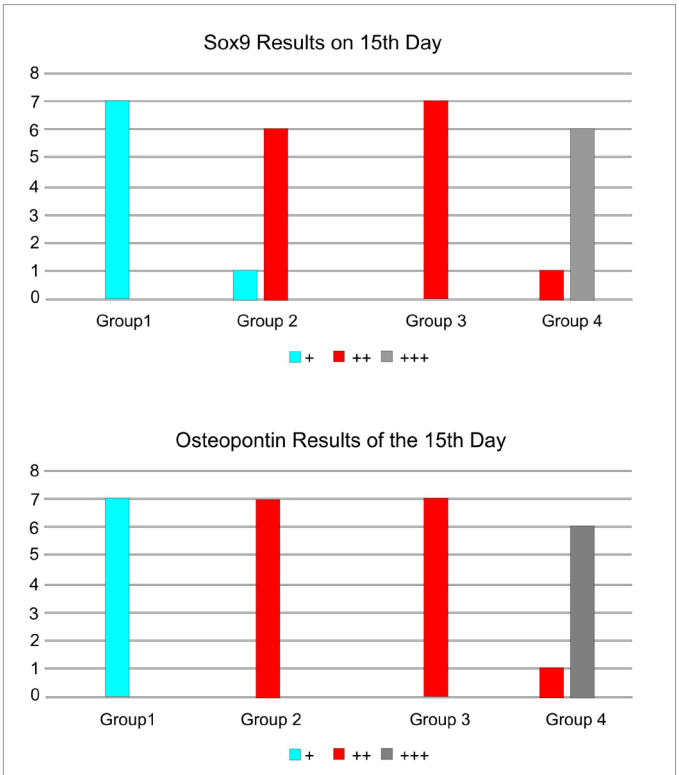

Figure 3. Early term SOX9 ve Osteopontin scores were shown with number of the rats

RESULTS

Body Weight Measurements

In order to ensure the accuracy of the study, the initial body weights of the rats were adjusted to be quite similar for all groups. No statistically significant differences were observed between the groups in the first, second, third, and fourth-week measurements (p values 0.992, 0.849; 0.936 and 0.916, respectively), (Table 2).

CT Measurements

Ten randomly selected CT measurements were repeated by the same researcher after 15 days to evaluate inter-examiner variability. No difference was seen in Pearson correlation coefficient (Table 3).

15th (T1) and 30th (T2) day measurements of Co-Gn, Co-Pg, Pg-Gn and Δ showed a significant increase compared to 1st day (T0). The dimensional changes in mandible showed no significant difference between the groups (Table 4).

SOX9 and Osteopontin

Early-term (15th day) SOX9 immunolocalization was higher in the experimental group than in the control group ($p<0.001$). Among the experimental groups, the most intensive immunolocalization was observed in Group 4 ($p<0.001$). There was no significant difference between Group 2 and Group 3 ($p>0.05$). Early-term (15th day) Osteopontin immunolocalization of experimental groups was more intensive than the control group ($p<0.001$). Similarly, the most intensive Osteopontin immunolocalization was observed in Group 4 ($p<0.001$). There was no significant difference between Group 2 and Group 3 ($p>0.05$) (Figure 3).

Late-term (30th day) SOX9 immunolocalization was significantly higher in the experimental group compared to the control group ($p<0.001$) (Figures 4 and 5). Among the experimental groups,

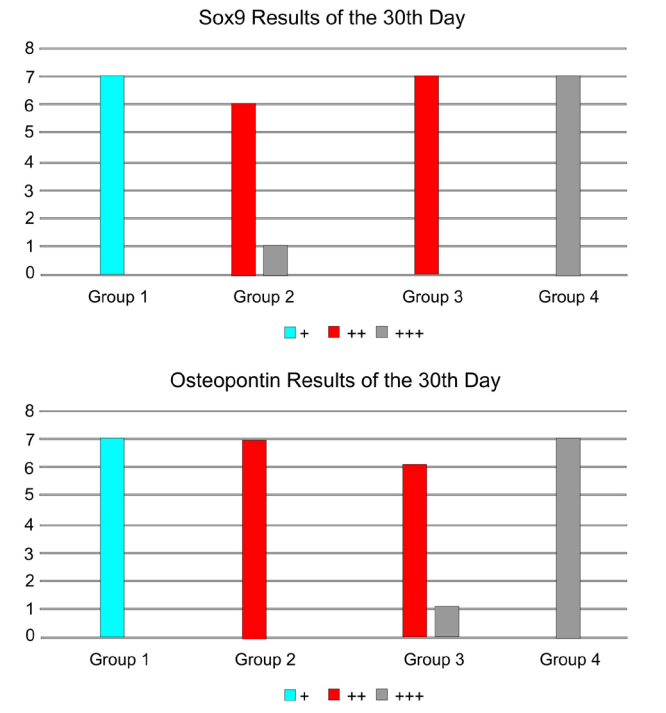

Figure 4. Late term SOX9 ve Osteopontin scores were shown with number of the rats

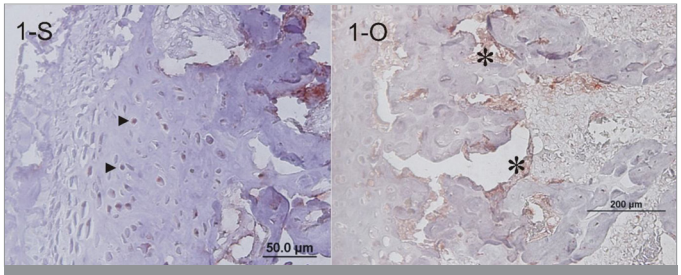

Figure 5. Histological images of Group 1 (control) S: SOX9, O: Osteopontin, *: new cartilage formation, *: new bone formation (mild level)

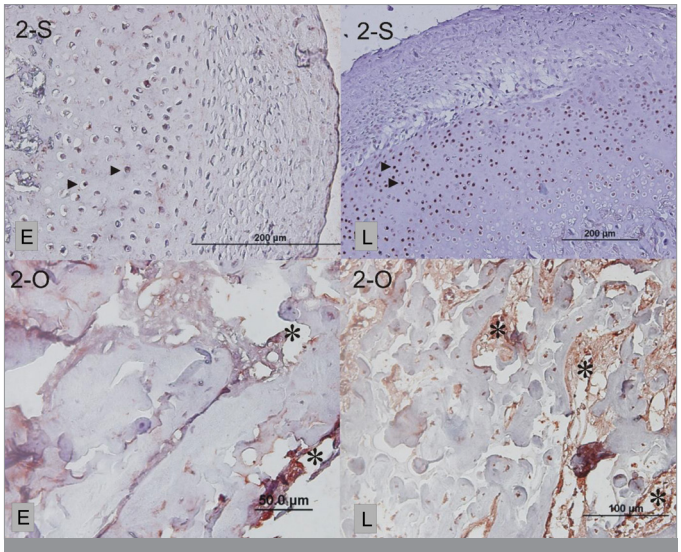

Figure 6. Histological images of Group 2 (SR). E: Early, L: Late, S: SOX9, O: Ostepontin, *: new cartilage formation, *: new bone formation (moderate level)

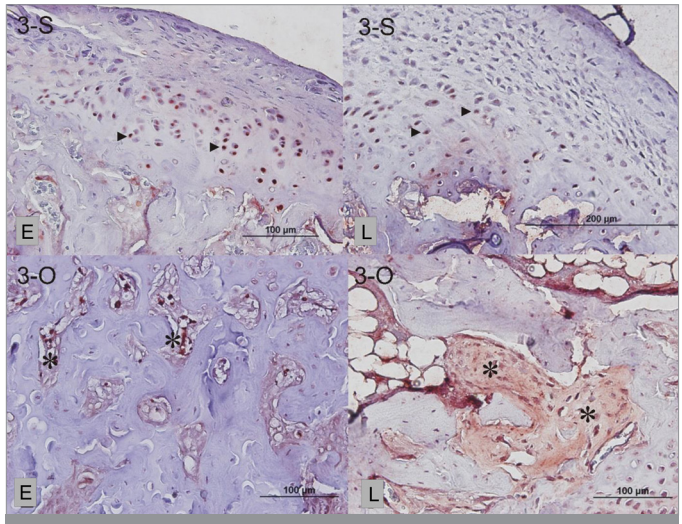

Figure 7. Histological images of Group 3 (MA). E: Early, L: Late, S: SOX9, O: Ostepontin, *: new cartilage formation, *: new bone formation (moderate level)

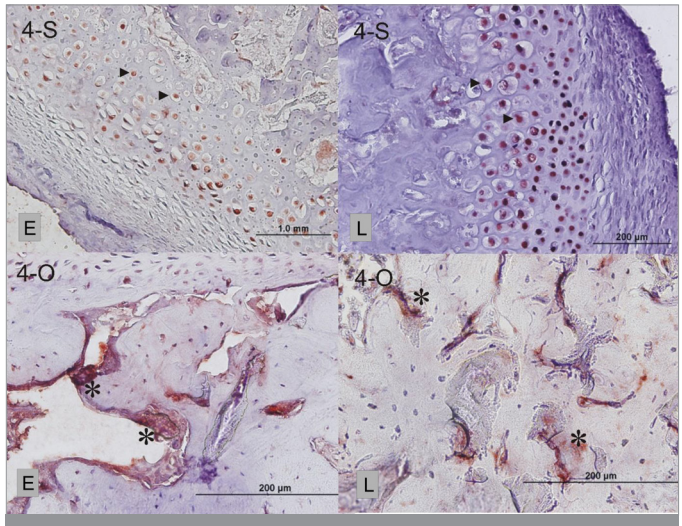

Figure 8. Histological images of Group 4 (SR+MA). E: Early, L: Late, S: SOX9, O: Ostepontin, 9: new cartilage formation, *: new bone formation (severe level)

the most intensive immunolocalization was observed in Group 4 ($p<0.001$). Group 2 and Group 3 immunolocalization levels were quite similar ($p>0.05$) (Figure 6 and 7). Late-term Osteopontin immunolocalization of experimental groups was more intensive than the control group ($p<0.001$). Similarly, the most intensive Osteopontin immunolocalization was observed in Group 4 ($p<0.001$) (Figure 8). There was no significant difference between Group 2 and Group 3 ($p>0.05$).

DISCUSSION

The main purpose of our experimental research was to evaluate the effect of systemic SR on condylar growth during mandibular advancement and to test whether it can be used therapeutically in the treatment of MA.

The age of the rats is crucial in such studies. Researchers selected 4-week or 8-week-old rats for similar experimental procedures

(16, 17, 21, 22) . However, in our opinion, 4-week old rats might not be strong enough to live with a bite jump appliance. Therefore we preferred to use 8-week-old rats. In another study mandibular advancement showed that condylar growth may occur even in 12-month or 18-month-old rats due to persistent chondrogenic cells (23).

Bite jumping appliance could be designed for rats using three methods: (1) two-piece appliance in both the lower and upper jaws, such a twin block; (2) a device only in the upper jaw; or (3) a device only in the lower jaw (18, 21, 24). Rat incisors are highly specialized for gnawing. They are open-rooted, which means they grow throughout life. The single lower jaw appliance was used in our study to ensure the success of the mandibular advancement and proper feeding of the animals.

Unlike other bisphosphonates, SR reduces bone resorption and simultaneously increases bone formation (25). SR improves the biomechanical properties and micro-architectural structure of the bones (26). However, other bisphosphonates have even been reported to reduce bone formation by up to 50% (27). The effective dose is very critical to accomplish the dual-effect of SR. Fuchs et al. (28) reported that the daily intake of SR at doses of 25 mg/kg or 150 mg/kg for 90 days was inadequate to prevent bone resorption and increase bone formation. The recommended daily effective human dose of SR is 2 grams. Bain et al. (29) stated that this dose corresponds to 625 mg/kg for rats. The discrepancy in dose among humans and rats is associated with low SR absorption in the gastrointestinal system of rats. In order to have antiresorptive effects, the daily SR intake of rats should be at least 308 mg / kg (25). SR was used in the previous two orthodontic studies at doses of 600 mg/kg and 900 mg/kg (12, 13). In our study a dose of 900 mg/kg was used to achieve a clear bone-building effect.

SR was used for experimental purposes in our study, but like any drug, SR has undesirable side effects. The most common side effects in clinical researches are (30): diarrhea, nausea, headache, and skin irritation. For patients with ischemic heart disease, uncontrolled hypertension, and cardiovascular system disorders, it has been stated that SR should not be used (31). As another side effect, the reduction of osteoclastic activity by SR may impair condylar growth. However, no such effects were observed in the histological findings. In further studies, this can be researched by the measurement of osteoclast activity markers.

The effect of mandibular advancement on condylar growth is still controversial. Some authors reported a minimal increase in condylar growth or total mandibular length (32, 33). However, other researchers have seen a significant increase in the mandibular dimension (34). McNamara et al. (35) found that mandibular advancement increased the mandibular length by 5-6 mm in monkeys. A rat mandible is much smaller than a monkey, so it is quite difficult to detect the condylar or mandibular dimensional change by CT measurement. The use of micro-CT instead of normal CT could have been more efficient. This could be the reason that in both early and late-term CT measurements, no difference was observed between all groups.

Condylar growth could be evaluated histologically using two methods: histomorphometric measurement or immunohistochemistry (36, 37). In the previous researches, the thickness of the condylar cartilage maturation, and proliferation layers was measured at three locations (anterior, middle, posterior) for histomorphometric evaluation (38). However, we only evaluated the ossification by a semi-quantitative scoring system in the middle of the condyle. The lack of histomorphometric measurements and lack of cell counts for quantitative assessment of the immunolocalization were limitations of our study.

Histological findings have shown that Osteopontin and SOX9 immunolocalization in Group 4 were significantly higher than in the other groups. Thus, we could say that a synergistic effect has occurred in the combination of mandibular growth and SR. Abtahi et al. (39) achieved the same synergistic effect by applying low-level laser (LLL) during mandibular advancement in rabbits. However, El-Bialy et al. (40) reported that there was no synergistic effect when LLL or light-emitting diode (LED) and MA were applied together. Furthermore, the use of a single LED or single LLL resulted in more bone formation than LED+MA or LLL+MA combinations. The mismatch between the findings of the two studies may have been related to testing different stimulating factors (SR and LLL or LED).

The bone and cartilage formation levels in Group 2 and Group 3 were similar. SR or MA protocol had the same cellular stimulation effect on the condylar cartilage. The results suggest that SR could stimulate condylar growth by itself or in combination with MA.

Early and late term results of ossification in condyle might be evaluated with a wide range of time intervals such as 3rd, 14th, 21st, and 30th days (18, 36). Our early-term histological findings were similar to the late-term findings of all groups. In the literature, there are different opinions that reported bone formation decreased or increased during the late period (18, 36, 41). We believe that the variety of evaluation methods leads to this disagreement.

CONCLUSION

SR is capable of stimulating condylar growth by itself. The stimulation effect could be further increased in combination with mandibular advancement. SR, which we used for experimental purposes, can not be used in orthodontic practice. However, in further studies, applications with little or no side effects could be tested.

Ethics Committee Approval: This study was approved by Ethics committee of Cumhuriyet University, (Approval No:17.03.2017- 25).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Supervision - H.C., C.D.; Design - H.C., C.D., S.S.; Supervision - H.C., C.D.; Resources - H.C.; Materials - H.C., C.D.; Data Collection and/or Processing - H.C.; Analysis and/or Interpretation - H.C., C.D., S.S. ; Literature Search - H.C.; Writing Manuscript - H.C., C.D.; Critical Review - H.C., C.D.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This research financed by Cumhuriyet University Scientific Research Project Committee.

Acknowledgements: The authors would like to thank financial support of Cumhuriyet University Scientific Research Project Committee.

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