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Original Article

The effect of local injection of the human growth hormone on the mandibular condyle growth in rabbit

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ABSTRACT

Background: The aim of this study was to evaluate the effect of local injection of human growth hormone (GH) in stimulating cartilage and bone formation in a rabbit model of temporomandibular joint (TMJ).

Materials and Methods: In an experimental animal study, 16 male Albino New Zealand white rabbits aged 12 weeks were divided into two groups: In the first group (7 rabbits) 2 mg/kg/1 ml human GH and in the control group (9 rabbits) 1 ml normal saline was administered locally in both mandibular condyles. Injections were employed under sedation and by single experienced person. Injections were made for 6 times with 3 injections a week in the all test and control samples. Rabbits were sacrified at the 20th day from the beginning of study and TMJs were histologically examined. ANOVA (two-sided) with Dunnett *post hoc* test was used to compare data of bone and cartridge thickness while chi-square test was used to analyze hyperplasia and disk deformity data. $P < 0.05$ was considered as significant.

Results: Cartilage layer thickness was greater in the GH-treated (0.413 ± 0.132) than the control group (0.287 ± 0.098) (*P* value = 0.02). Although bone thickness and condylar cartilage hyperplasia were greater in the GH-treated group, these differences were not statistically significant (*P* value = 0.189 and 0.083, respectively). There was no statistically significant difference between two groups regarding the disc deformity (*P* value = 0.46).

Conclusion: Local injection of human GH in the TMJ is able to accelerate growth activity of condylar cartilage in rabbit.

Key Words: Growth, human growth hormone, mandibular condyle, rabbits

INTRODUCTION

Of all the problems that fall into the dentofacial deformity category, mandibular deficiency is the most prevalent that is usually treated with functional appliance in a preadolescent child and with orthognathic surgery or distraction osteogenesis in adults.[1] As a general guideline, even in the most favorable circumstances, it is unlikely that more than half of the long-term changes needed to correct Class II malocclusion would be gained by differential jaw growth.[2] In addition, unwanted tooth movement, poor biomechanical control, and lack of patient cooperation often lead to unsuccessful growth modification with functional appliances. Orthognathic surgery may also cause some complications in both bones and soft tissues after the operations, including bone necrosis, temporomandibular joint (TMJ) problems, scars, and nerve injury.[3] Therefore, a novel strategy for both children and adults with fewer disadvantages is a clear need. It is postulated that medical intervention in the TMJ by one of the most efficient hormones that is growth hormone is a novel therapy for mandibular deficiency for both children and adults. However, there have been few research works into medical methods aimed at controlling

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condylar growth.[4-8] The condylar cartilage is as a significant growth site of the mandible as is the epiphyseal cartilage in the long bones. However, this cartilage is not the same as the epiphyseal cartilage in many aspects. First, the condylar cartilage shows a unique mode of cell proliferation and differentiation. The condylar cartilage does not form columns of proliferating chondrocytes and grows multi-directionally to match to the mandibular fossa of the temporal bone. Second, the cartilage responds in a different way to humoral factors and mechanical loads.[9,10] Moreover, the condylar cartilage does not disappear even after its full development so it can adapt to various functional demands. Therefore, the control of cartilage development is serious for the overall growth of the mandible and occlusion.[11-13] This study examined the effects of human growth hormone (HGH) on the growth of the condylar cartilage. GH, which is secreted by the pituitary gland, plays an important role in longitudinal bone growth. Although GH stimulates increased deposition of protein and increased growth in almost all tissues of the body, its most obvious effect is to increase growth of the skeletal frame. GH produces much of its effect through intermediate substances called somatomedins. Even so, experiments have shown that injection of GH directly into the epiphyseal cartilage of bones of living animals causes specific growth of cartilage areas.[14] It has been reported that GH produces a strong stimulatory effect on progenitor cell proliferation, cartilage differentiation, and extracellular mineralization, which supports *de novo* bone formation in vitro.[15] According to Ramirez-Yanez *et al*., GH stimulates mitotic activity and delays cartilage cells maturation in the mandibular condyle. This effect at the cellular level may produce changes in the cartilage thickness.[16] It is known that GH therapy primarily affects craniofacial regions where cartilage-mediated growth occurs and regions that adapt to cartilage growth-particularly the mandibular ramus.[17,18] However, GH therapy is indicated in actual GH deficiency; and there has been much debate regarding the effects of systemic HGH administration in patients who do not have GH deficiencies. Furthermore, side effects of GH therapy include carpal tunnel syndrome, increased risk of diabetes, immune response to hormone and even malignancies control its systemic application in patient with no hormone deficiency.[19] It finally seems that local administration of GH could be more reasonable due to its more specific effects and less systemic side effects. It was the aim of the present study to test the effect of local injection of GH on mandibular condyle cartilage and bone thickness, cartilage hyperplasia, and disk deformity.

MATERIALS AND METHODS

Sixteen normal male New Zealand white Albino rabbits aged 12 weeks were used in the experiment. Rabbits had average weight of 1.6 kg and were housed in separate metal cages at a temperature of 23 ± 2°C and a relative humidity of 40 ± 5%. Animals were divided into two groups. Seven rabbits were injected intra-articularly with 2 mg/kg body weight of recombinant HGH (Eutropin, 601 Yongie-dong Iksan-si, Jeonbuk, South Korea) in the first group. In the second group (9 rabbits), the TMJs were injected with 1 ml sterile saline solution similarly to determine whether the trauma from injection might account for any change in growth or joint morphology. The animals were allowed free access to tap water and were fed a commercial rabbit diet provided from Razi institute. All phases in this experimental animal study were done in accordance with the ethical standards recommended by the American Animal Care and Use Committee.[20] Injections were employed under sedation induced by intraperitoneal Acepromazine 2% (Neurotranq, Alfasan, Woerden, Holland) and ketamine 10% (Alfasan, Woerden, Holland) and by single person. The TMJ areas of all rabbits were cleaned and sterilized. A sterile technique was applied to all intraarticular injections. A technique for correct injection into the TMJ was developed in a pilot study by injecting coloured solution in four animals. The correct position of the needle tip was confirmed during injection by palpation of condylar movement while the mandible was manually forced from one side to other side and then by surgical exposure of that site. Recombinant human GH was (2 mg/kg/ in volume of 1ml) administered in the test group every other day for 12 days and 1 ml sterile normal saline (Sodium chloride 0.9%, ghazi pharm, Tabriz, Iran) was similarly injected in the control group. No post-operative medications were given. All animals were euthanized on the 8th day after final administration with vital perfusion method. Both TMJs were taken en bloc and fixed in 10% buffered formalin. The specimens were decalcified and embedded in paraffin. The blocks were serial-sectioned sagittally to get to the center of the joint. The prepared 5-μm thickness sections were stained with hematoxylin and eosin. Magnified photographs (×100 and ×400) of the

lateral view of the TMJs were obtained by a digital camera (Nikon DP-12 Camera) registered with a light microscope (light microscope Nikon YS 100, Tokyo, Japan) to allow for the linear measurements. Bone and cartilage thickness were measured using Image-Analysis software (Nilu Pathology Image Analyzer version 1.0). Total thickness of the cartilage was determined by measuring the distance from the superior border of the mandibular cartilage to boundary with the zone of endochondral ossification. In this study, measurements were performed with no knowledge of treatment by an observer. Two-way ANOVA with Dunnett *post hoc* test was used to compare data of bone and cartridge thickness while the chi-square test was used to analyze hyperplasia and disk deformity data. $P < 0.05$ was considered as significant.

RESULTS

Generally, no adverse effects were found by vehicle and/or drug treatments. The mean weight gain was 100 g and did not differ significantly between experimental and control groups ($P > 0.05$). All measurements are summarized in Tables 1 and 2. Cartilage thickness measured on sections stained with hematoxylin and eosin was significantly greater in the rabbits treated with GH (mean ± standard deviation = 0.413 ± 0.132), compared with the control group (0.287 ± 0.098) ($P < 0.05$) [Figures 1 and 2]. Great but not statistically significant difference in bone thickness was observed between test (0.72 ± 0.174) and the control (0.6 ± 0.18) specimens ($P > 0.05$). Indeed, a relatively greater increase in bone thickness was noted in the test group compared with controls; however, this difference was not significant. Condylar hyperplasia was observed in 64.3% and 33% of test and control groups, respectively; however this substantial difference was not statistically significant [Figures 3 and 4]. The effects of local injections of GH on disc deformity were not statistically significant. Disc deformity was observed in 14% and 22.2% of test and control group, respectively [Figures 5 and 6].

DISCUSSION

In the present study, effects of local administration of GH on cartilage and bone thickness, cartilage hyperplasia, and disk deformity were measured in the mandibular condyle of rabbits. Direct effects of GH on growth plate cartilage[21,22] and on mandibular cartilage from neonatal animals[15,16] were described in advance. Hunziker *et al*.[23] alleged that both IGF-I and GH exert their influence on chondrocytes at each stage of differentiation, rather than acting specifically upon particular subpopulations of cells at some phases of differentiation. Eden *et al*.[24] showed that GH has

Table 1: All measurements of right and left temporomandibular joints of seven rabbits that were treated with 2 mg/kg/1 ml recombinant human GH every other day for 12 days

Number	Bone Thickness	Cartilage Thickness	Disk Deformity (Yes/No)	Cartilage Hyperplasia (Yes/No)
G.H R1	0.66	0.33	–	+
G.H L1	0.66	0.33	–	+
G.H R2	0.33	0.246	+	–
G.H L2	0.576	0.33	–	–
G.H R3	0.59	0.33	–	–
G.H L3	0.82	0.246	–	–
G.H R4	0.49	0.33	–	+
G.H L4	0.91	0.46	–	+
G.H R5	0.82	0.496	–	+
G.H L5	0.91	0.44	–	+
G.H R6	0.825	0.66	–	+
G.H L6	0.77	0.66	–	+
G.H R7	0.82	0.44	–	+
G.H L7	0.9	0.49	+	+

Table 2: All measurements of right and left temporomandibular joints of nine rabbits that were treated with 1 ml sterile normal saline every other day for 12 days as the control group

Number	Bone Thickness	Cartilage Thickness	Disk Deformity (Yes/No)	Cartilage Hyperplasia (Yes/No)
Control R1	0.69	0.25	–	–
Control L1	0.82	0.33	+	+
Control R2	0.49	0.246	–	–
Control L2	0.49	0.11	–	+
Control R3	0.66	0.33	–	–
Control L3	0.66	0.33	+	–
Control R4	0.33	0.165	–	–
Control L4	0.66	0.41	–	+
Control R5	0.33	0.17	–	+
Control L5	0.33	0.165	–	+
Control R6	0.89	0.246	–	–
Control L6	0.91	0.246	–	–
Control R7	0.44	0.33	–	–
Control L7	0.576	0.246	–	–
Control R8	0.66	0.44	–	–
Control L8	0.66	0.33	–	–
Control R9	0.495	0.396	+	–
Control L9	0.83	0.44	+	+

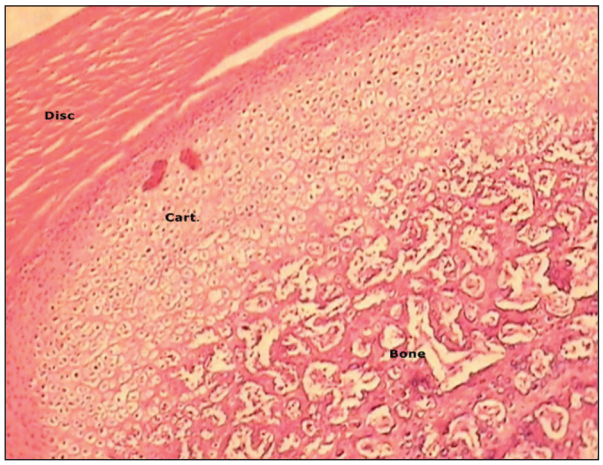

Figure 1: Photomicrograph of temporomandibular joint of a rabbit treated with 2 mg/kg/1 ml recombinant human GH every other day for 12 days (Sagittal section. Hematoxylin & eosin staining. Magnifications: ×100)

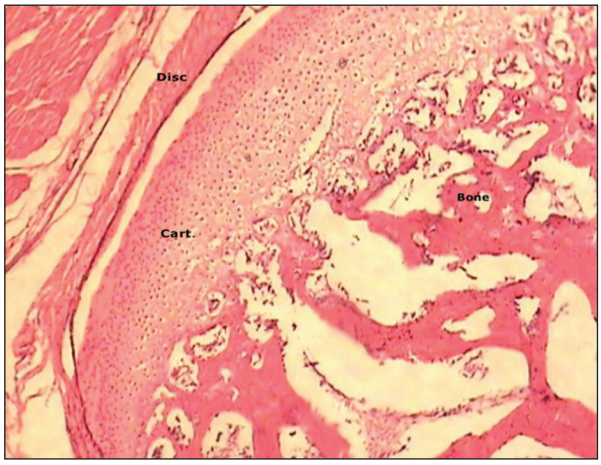

Figure 2: Photomicrograph of temporomandibular joint of a rabbit treated with 1 ml sterile normal saline every other day for 12 days (Sagittal section. Hematoxylin & eosin staining. Magnifications: ×100)

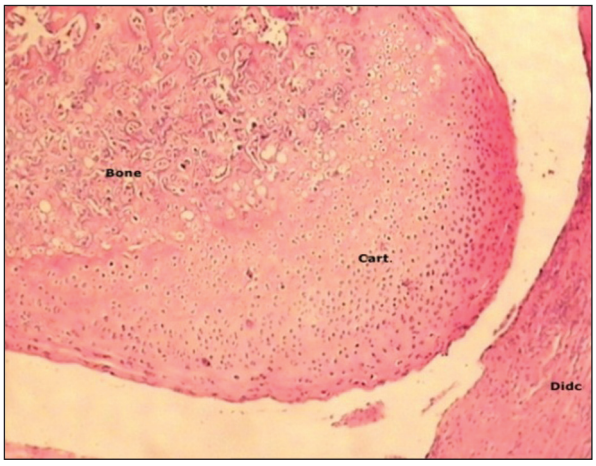

Figure 3: Photomicrograph of temporomandibular joint. Note to cartilage hyperplasia (Sagittal section. Hematoxylin & eosin staining. Magnifications: ×100)

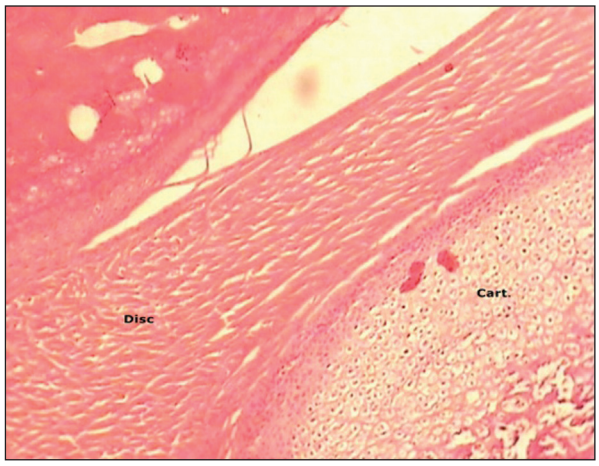

Figure 4: Photomicrograph of temporomandibular joint. Note to small proliferative layer (Sagittal section. Hematoxylin & eosin staining. Magnifications: ×100)

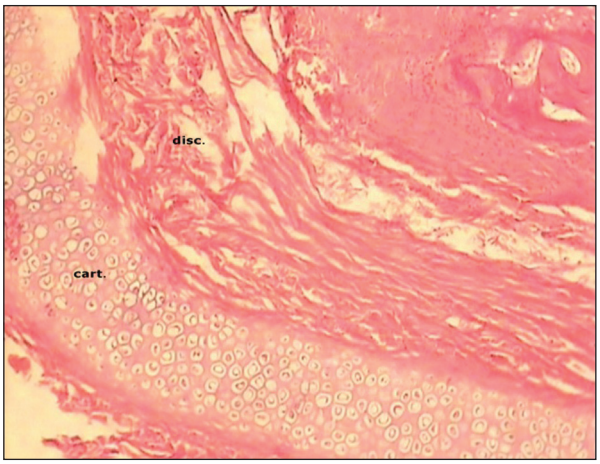

Figure 5: Photomicrograph of temporomandibular joint. Note to disk deformity (Sagittal section. Hematoxylin & eosin staining. Magnifications: ×100)

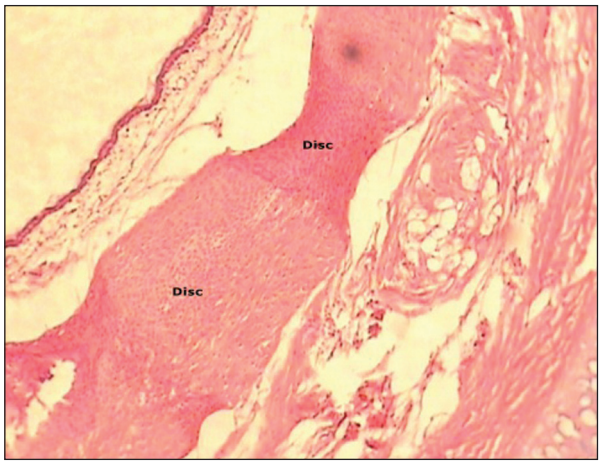

Figure 6: Photomicrograph of temporomandibular joint. Note to normal disk (Sagittal section. Hematoxylin & eosin staining. Magnifications: ×100)

a direct influence on stimulation and proliferation of chondrocytes.

Using the present animal study, it became apparent that GH possesses a marked stimulatory effect on the cartilage growth; rabbits treated with 2 mg/kg GH demonstrated significantly higher cartilage thickness as it has been shown *in vitro* in mandibular condylar explants,[15,25-27] and *in vivo* in mandibular condylar cartilage.[28-31] Interestingly, Luder *et al* showed that the cartilage thickness is significantly reduced in the dwarf animals treated with GH. They came to the conclusion that increased cell volume and higher rates of matrix synthesis in the hypertrophic layer contribute to cartilage thickness three or four times more than the proliferative and the mature layers.[32] In the dwarf rats treated with GH, cell proliferation is stimulated, the number of cells in the proliferative layer increased and therefore increasing the thickness of this layer. However, these are flattened cells and an increase in the proliferative layer does not significantly change the total cartilage thickness. Chondrocytes maturation is postponed in these animals, which means lower numbers of cells are going through the mature layer into the hypertrophic layer. Accordingly, there are diminished numbers of cells of high volume secreting cartilage matrix. Thus, the reduction in the cartilage thickness seen in the dwarf rats treated with GH is explained by a reduction in the numbers of cells differentiating, maturing, and reaching the hypertrophic layer.

Although there is a large body of experimental evidence indicating that GH effects on chondrocytes are mediated by IGF-I, it is not perhaps an exclusive pathway. The possibility has not been excluded that in the present study, GH may have induced IGF-I synthesis, and that the effects shown may have been because of IGF-I and not as a result of a direct effect of GH.

The data shows that bone thickness as determined by the linear measurement is increased in the rabbits treated with GH; however, this substantial difference was not statistically significant. Increased bone formation with GH administration has been confirmed with previous reports that GH was found to stimulate endochondral bone formation.[25,33,34] According to Livne *et al.,*[26] tissue sections from cultures of animals treated with GH showed dark staining along the cartilage-bone interface and exposure to tetracycline indicating enhanced mineralization in this region. Ehrnberg *et al*. showed that GH induces an increase in bone formation, bone mineral content, and maximum torque capacity of the diaphyseal bone during 1 to 2 months.[35]

Although cartilage hyperplasia was substantially greater in animals treated with GH, this difference was not statistically significant; however, it is known that GH promotes increased size of the cells and increased mitosis with development of increased number of cells and specific differentiation of certain types of cells such as bone growth cells.[14] In humans, GH treatment has been shown to enhance mandibular growth.[36] Stimulation in mitotic activity results in more cartilage cells being accessible to differentiate into chondrocytes from the mesenchymal cells. Thus, a higher number of cells will be depositing extracellular matrix which later will turnover into bone.[32] In this way, GH would be responsible, at least in part, for increases in length of the mandible.

Another purpose of this study was to examine the ability of GH to enhance joint abnormalities resulted from needle trauma because it has been proposed that GH replacement therapy reverses most of abnormalities in joint physiology in patients with both GH deficiency and/or excess.[37] According to the results we obtained in the present study, there was lower disk deformity in rabbits treated with GH than the control group. This might be related to GH ability in repair and regeneration of collagenous tissues although the difference between two groups was not significant.

CONCLUSION

This study represents the effect of local administration of GH on the mandibular condylar cartilage of rabbits over 20 days. Histological changes, such as an increase in the thickness of cartilaginous layers and bone thickness, were observed in the GH-treated group; although the latter was not statistically significant. GH treatment stimulates cartilage hyperplasia, but does not affect the prevalence of disk deformity.

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