J Med Dent Sci 2003; 50: 79–85

Original Article

Effects of local administration of insulin-like growth factor-I on mandibular condylar growth in rats

Kouichi Itoh, Shoichi Suzuki and Takayuki Kuroda

Maxillofacial Orthognathics, Maxillofacial Reconstruction Division of Maxillofacial/Neck Reconstruction Graduate School, Tokyo Medical and Dental University

Endochondral bone formation observed at the mandibular condyle is regulated by various growth factors including insulin-like growth factor-I (IGF-I). In this paper, we describe a method for the local administration of IGF-I to the bilateral mandibular articular cavities of 3- and 12-week-old rats, and the effects of IGF-I on endochondral bone formation by histomorphometric techniques.

In 3-week-old IGF-I-treated rats, three days after administration, an increase in bone tissue was found in the area of the subchondral cancellous bone layer.

In 12-week-old IGF-I-treated rats, three days after administration, an increase in the thickness of the condylar cartilage and a decrease in bone tissue were observed in the area of the subchondral cancellous bone layer.

This study revealed that the local administration of IGF-I on mandibular condyle caused different histological changes between growth and maturation periods. These results indicated that the effects of IGF-I on endochondral bone formation in the mandibular condyle were age-dependent.

Key words: insulin-like growth factor-I, mandibular condyle, endochondral bone formation, rat, local administration

Corresponding Author: Kouichi Itoh, DDS
Maxillofacial Orthognathics, Maxillofacial Reconstruction, Division of Maxillofacial/Neck Reconstruction, Graduate School, Tokyo Medical and Dental University.
5-45, Yushima 1-chome, Bunkyo-ku, Tokyo 113-8549, Japan.
Tel 03-5803-5538 Fax 03-5803-0203
E-mail k-itoh.mort@tmd.ac.jp
Received October 18, 2002; Accepted January 14, 2003

Introduction

Insulin-like growth factor-I (IGF-I) is produced and secreted in the liver and other local tissues upon stimulation by growth hormone, and promotes the growth of various tissues such as bone and cartilage.[1,2] According to previous studies, IGF-I contributes to the growth of longitudinal bones by stimulating endochondral bone formation in the growth plate.[3,4]

Recently, several roles of IGF-I in the growth of mandibular condyle have been examined. Maor *et al*.[5-7] reported that IGF-I facilitated the proliferation of cartilaginous cells in mouse condyle *in vitro* by adding IGF-I to the culture medium. Furthermore, Visnapuu *et al*.[8] revealed the distribution of IGF-I receptors in the mandibular condyle of rats by immunohistological and *in situ* hybridization experiments. These results indicated that IGF-I probably also played important roles in the growth of the mandibular condyle.

Several previous reports have shown that local or systemic extrinsic administration of IGF-I caused histological changes in the growth plate, and enhanced long axial growth of longitudinal bone.[9-14] However, to our knowledge, there have been no similar studies on mandibular condylar cartilage *in vivo*.

The purpose of this study was to establish a method for local administration into the mandibular condyle of rats, and to investigate the histological changes in the condyle after the local administration of IGF-I.

Materials and methods

Hormones and chemicals

Bacteria-derived human recombinant insulin-like growth factor I (rhIGF-I, R & D Systems, Minneapolis, MN) was dissolved in physiological saline to a concentration of 20 μg/ml. It was the lowest dose which could observe histological changes in our earlier study. Tetracycline and calcein were purchased from Wako Pure Chemicals, Osaka, Japan.

Animals

Thirty 3-week-old and 20 12-week-old male Sprague-Dawley rats (Sankyo Lab Service Co., Inc., Tokyo, Japan) were used in this study. They were divided into control and treatment groups and weighed once a week.

Procedure for administration

The animals were anesthetized intraperitoneally with sodium pentobarbital. After the articular capsule of the temporomandibular joints was exposed, the tip of a needle (27-gauge 0.75-inch, Terumo, Tokyo, Japan) on a tuberculin syringe was inserted into the articular capsule, and the drug solution was injected slowly. The animals in the treatment group were administered 0.02ml saline solution of IGF-I. The animals in the control group were administered an equivalent volume of physiological saline. Ten 3-week-old male Sprague-Dawley rats were used for a fluorescent-labeling study. Two hours before the administration of IGF-I or saline, each rat was given 8 mg/kg tetracycline intraperitoneally for the fluorescent labeling of bone. They were killed on the third day after administration. Five hours before killing, a second fluorescent label, calcein (3 mg/kg), was given intraperitoneally. On the third, fifth and seventh days after administration, 3-week-old rats were killed with an overdose of sodium pentobarbital, while 12-week-old rats were killed in the same way on the third and fifth days. All procedures used in this experiments were approved by an institutional committee on animal experimentation.

Preparation of sections and histomorphometric measurements

After killing, the mandibular condyles were dissected out and immersed in Karnovsky's fixative solution (pH 7.2) for three days.

The condyles on the left side were decalcified with 2% formic acid for one week and embedded in glycolmethacrylate resin (Historesin, Leica Microsystems, Nussloch, Germany). Decalcified ground sections were then prepared and used for histological observation. We followed the example of Noguchi[15] in defining the zones of the cartilage (Fig. 1).

The condyles on the right side were dehydrated through a series of increasing concentrations of ethanol (to 100%), filtered, and embedded in acrylic resin (LR white resin, The London Resin Co. Ltd., UK). Before being embedded in acrylic resin, three reference points (A, the anterior edge between the cartilage and bone; B, the posterior edge between the cartilage and bone; and C, the midpoint of A and B on the uppermost articular surface in the sagittal dimension) were marked directly points could be defined under the microscope, the coordinate axis (X-axis, line AB; Y-axis, perpendicular line through the midpoint of AB) in each section could be easily aligned (Fig. 2a, b). The condyles on the left side were decalcified with 2% formic acid for one week and embedded in glycolmethacrylate resin (Historesin, Leica Microsystems, Nussloch, Germany) on each condyle using white paint. The tissue block was trimmed with a microtome so that the three reference points could be lined up on the same plane. The oriented plane of the tissue block was bonded to the plastic slide glass and ground manually. The thickness of the ground section was 50-70 μm.

Undecalcified ground sections were used for fluorescent microscopy. The growth rate of endochondral bone formation could be calculated by measuring the distance between the calcein and tetracycline labels (Fig. 2a).

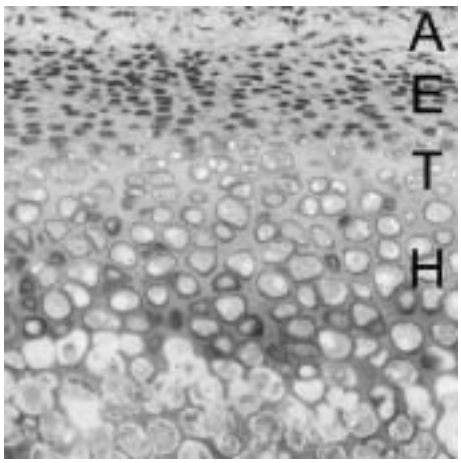

Fig. 1. Cellular organization of the mandibular condyle of rat. A, articular zone; E, embryonic zone; T, transitional zone; and H, hypertrophic zone.

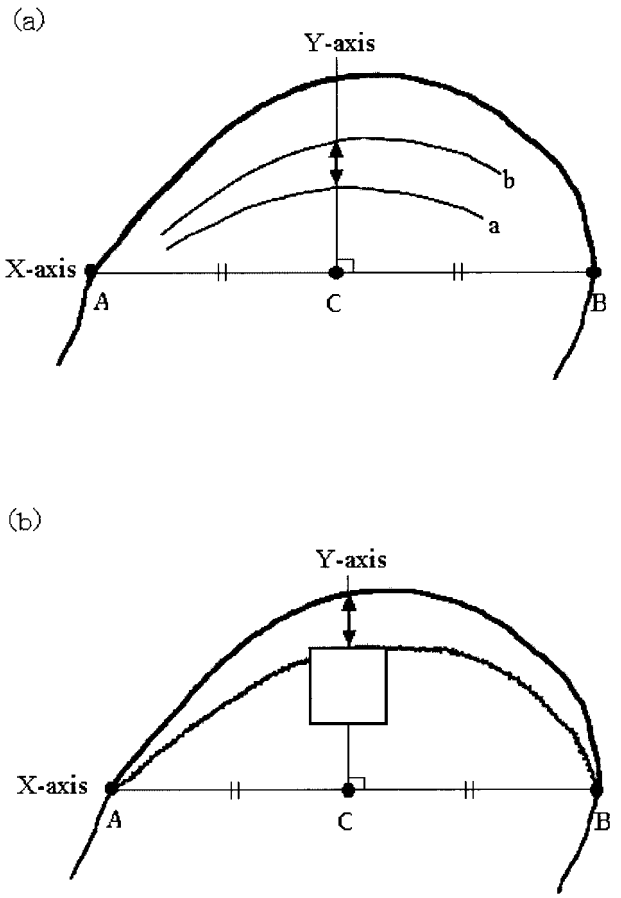

Fig. 2. a) Schematic drawing of section from mandibular condyle. Point A, the anterior edge between the cartilage and bone; Point B, the posterior edge between the cartilage and bone; and Point C, the midpoint of A and B on the uppermost articular surface in the sagittal dimension; X-axis, line AB; Y-axis, perpendicular line through the midpoint of AB; line a, fluorescent label of tetracycline; line b, fluorescent label of calcein. Vertical arrow indicated the measurement of Interlabel width of the double fluorescent labels. b) Schematic drawing of section from mandibular condyle. Vertical arrow indicated the thickness of the cartilaginous layer of the condyle. Measurement of bone area was made in the black square.

After the growth rate was calculated, undecalcified sections were stained with a 1.0% aqueous solution of Azure A (pH 5.4) and counterstained with a 0.7% aqueous solution of Toluidine blue O (pH 6.8).

The thickness of the cartilaginous layer was measured along the Y-axis using a micrometer (Fig. 2b). To evaluate the activity of bone formation in the subchondral cancellous bone layer, the ratio of bone area to the total tissue (percentage of the bone area) within a square (0.5×0.5 mm) was calculated under magnification (×20) using image-analysis software (Mac Scope, Mitani Corporation, Tokyo, Japan). The square was positioned so that its vertical edge was parallel to the Y-axis of the section, and its upper horizontal edge was placed on the upper edge of the cartilage lacuna which initially opened to the bone marrow (Fig. 2b).

To statistically examine differences in the increase in body weight, the growth rate of endochondral bone formation, the thickness of the cartilaginous layer and the percentage of bone area in the subchondral cancellous bone layer between the IGF-I and control groups, Student's t-test was performed using Stat View (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

Body weight

No significant difference in the increase in body weight was found between the control and IGF-I groups at 3 or 12 weeks of age.

Histological observation

In the IGF-I group at 3 weeks of age, three days after administration, more osteogenesis was observed around the calcified cartilage spicules in the subchondral cancellous bone area than in the control group (Fig. 3a, b). However, seven days after administration, no significant difference was seen in the subchondral cancellous bone area.

In the IGF-I group at 12 weeks of age, three days after administration, a significant increase in the thickness of the cartilaginous layer, especially the thickness of the hypertrophic chondrocyte layer, was seen and the area of the subchondral cancellous bone layer in each bone spicule was reduced (Fig. 3c, d).

Thickness of the cartilaginous layer of the condyle

There was no significant difference between the control and IGF-I groups at 3 weeks of age (Fig. 4a). In the IGF-I group, at 12 weeks of age, the thickness of the cartilaginous layer was increased to 125% of the control group at three days after administration. However, it then decreased to the control level by five days after administration (Fig. 4b).

Growth rate of endochondral bone in 3-week-old rats

The average growth rate of endochondral bone in the IGF-I group was 0.28 mm/day, while that in the control

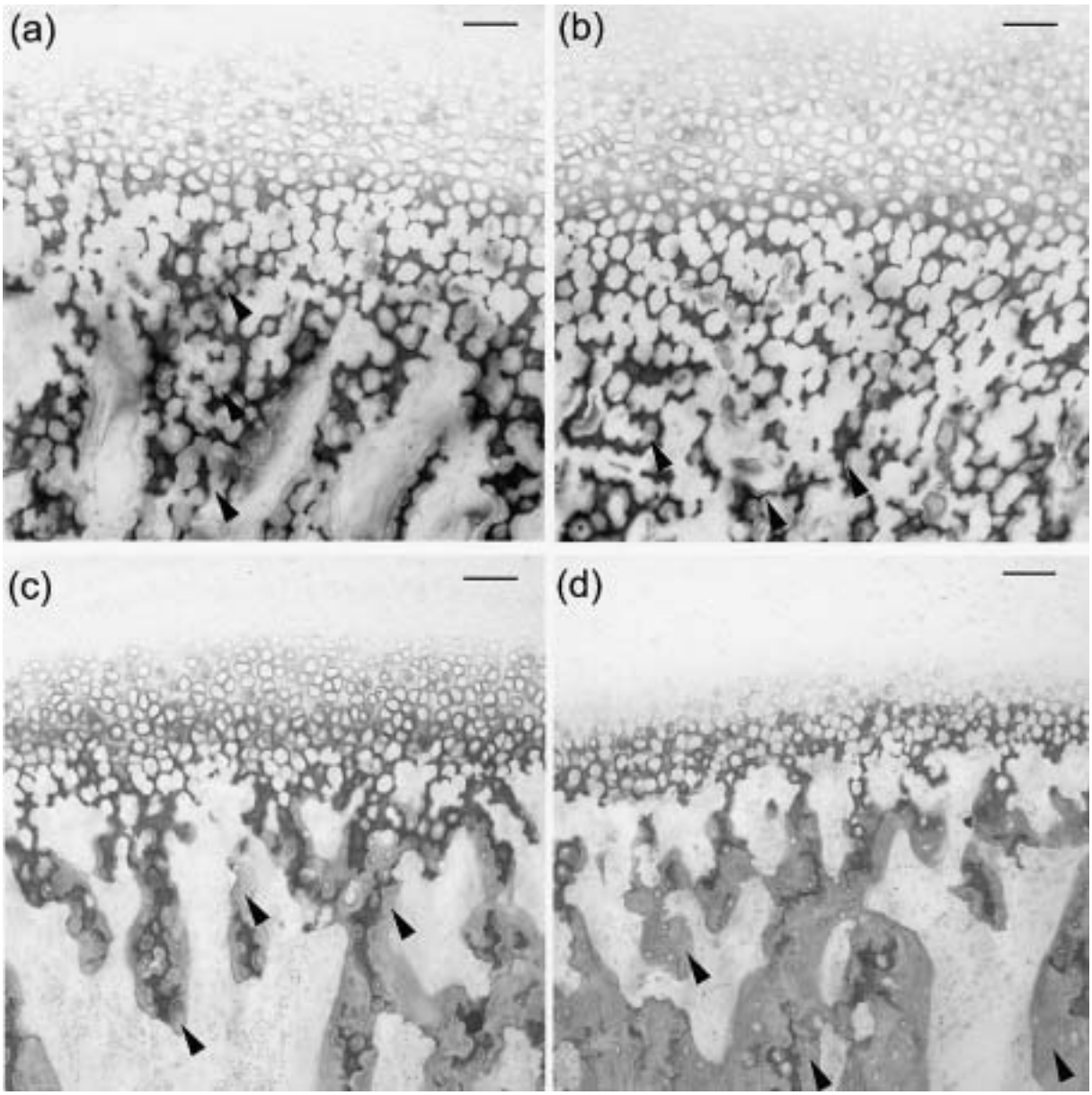

Fig. 3. Area of the subchondral cancellous bone layer in the mandibular condyle. a) 3-week-old rat at 3 days after IGF-I administration; b) 3-week-old control rat at 3 days after administration; c) 12-week -old rat at 3 days after IGF-I administration; d) 12-week-old control rat at 3 days after administration. Arrowhead, bone tissue in the subchondral cancellous bone layer; bar = 100 μm.

group was 0.29 mm/day. This difference was not significant.

Measurement of bone area in the subchondral cancellous bone layer

In the control group, at 3 weeks of age, the percentage of bone area in the subchondral cancellous bone layer slightly increased with time. In the IGF-I group, the percentage of bone area in the subchondral cancellous bone layer showed a distinct increase at three days after administration, and then decreased to the control level by seven days after administration (Fig. 5a). In the control group at 12 weeks of age, the percentage of bone in the subchondral cancellous bone layer was approximately 33%, and this did not change during the experimental period. In the IGF-I group, the percentage of bone decreased to 65% of the control group at three days after administration, and then

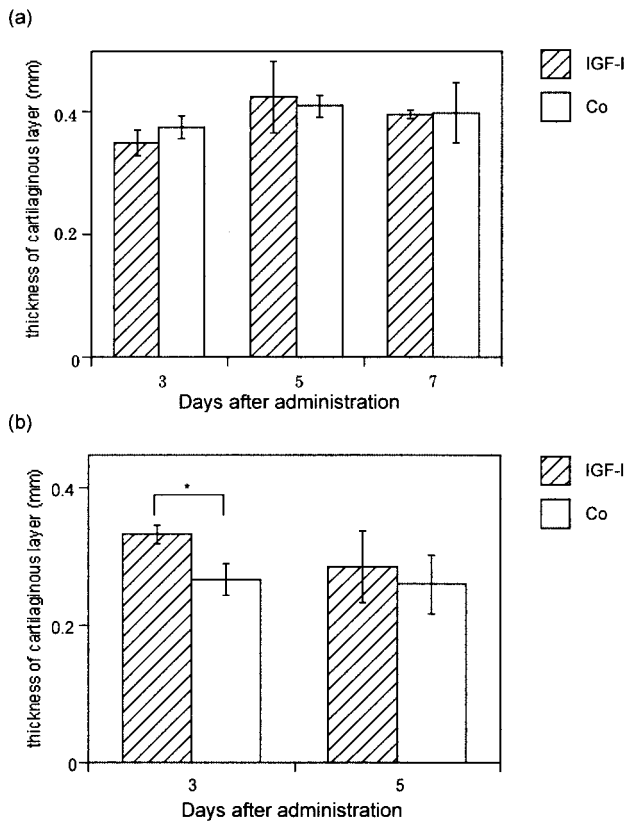

Fig. 4. Change in the thickness of the cartilaginous layer in 3-(a) and 12-week-old rats (b) treated with either IGF-I or physiological saline solution. In the control group, there was no significant difference. In the IGF-I group, at 12 weeks of age, the thickness of the cartilaginous layer was increased to 125% of the control group at three days after administration. *: $p<0.01$ (n=5 for each group).

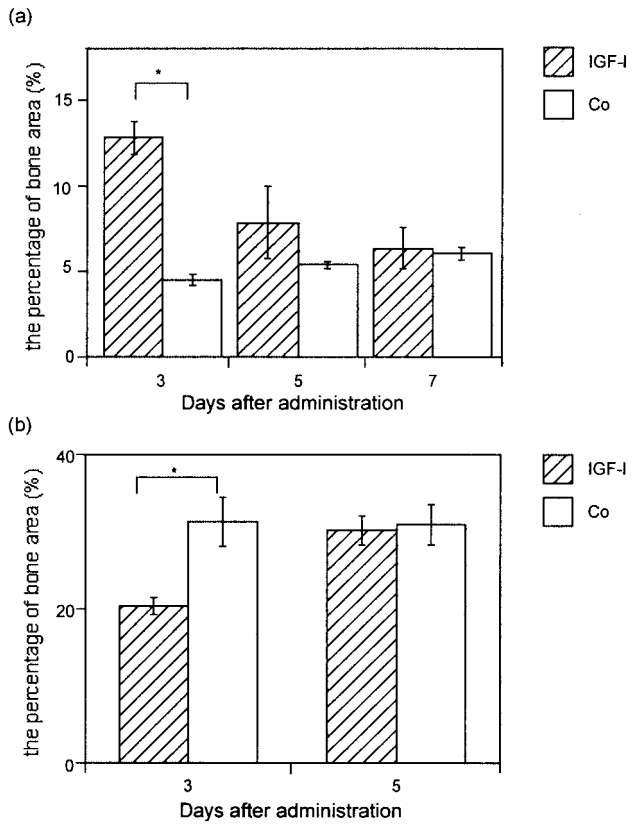

Fig. 5. Change in the percentage of bone area in the subchondral cancellous bone layer in 3-(a) and 12-week-old rats (b) treated with either IGF-I or physiological saline solution. In the control group, at 3 weeks of age, the percentage of bone area in the subchondral cancellous bone layer slightly increased with time (not significant), and, at 12 weeks of age, there was no significant difference. In the IGF-I group, at 3 weeks of age, the percentage of bone area showed a distinct increase at three days after administration and, at 12 weeks of age, the percentage of bone decreased to 65% of the control group at three days after administration. *: $p<0.01$ (n=5 for each group).

recovered to the control level by five days after administration (Fig. 5b).

Discussion

The mandibular condyle is one of the main growth sites of the mandible and is located on both ends of the mandible.[16-21] The condylar head is covered with the articular capsule, and the embryonic cellular layer supplying cartilaginous cells is immediately under the perichondrium in the articular surface of the condyle. Therefore, the administration of drugs into the articular cavity should be useful for accessing immature cells in the embryonic zone. Considering these condylar structures anatomically, the condyle is extremely advantageous for the local administration of drugs. Furthermore, the method used in this experiment was shown to be simple and effective.

In previous studies, where IGF-I or growth hormone was administered to the growth plate of longitudinal bone, the pituitary gland was excised from the experimental animals beforehand.[9-11,22,23] Since the aim of these studies was to investigate the direct effect of growth factors on tissues, it was necessary to eliminate the influence of endogenous growth factors. However, further purpose of our study was to obtain the therapeutic effects of the local administration of growth factors on the condylar growth of the patient. Therefore, we did not remove the pituitary gland in our study to investigate the influence of the IGF-I under normal hormonal condition.

Suzuki[24] measured the amount of condylar growth in

3- to 10-week-old rats using a vital stain method, and reported that the amount of growth was greatest in 3-week-old rats: approximately 1.5 times greater than that in 6-week-old rats, and 4.5 times greater than that in 10-week-old rats. Thus, the construction of tissue in the condyle during the growth period probably differs from that during maturation. Durkin *et al*.[25] distinguished the characteristics of the condylar cartilage in mature rats from those in growing rats, and reported that they responded differently to external stimulation. At the completion of condylar growth, the replacement of cartilage with bone by endochondral bone formation is terminated, and the condylar cartilage changes from growth cartilage to articular cartilage. Therefore, in this study, by establishing two stages of different ages (growth and maturation periods), the influence of the local administration of IGF-I on tissue in each period was examined using histomorphometric methods.

In this study, the percentage of bone area in the subchondral cancellous bone layer was slight, but gradually increased in 3-week-old control rats. However, in the IGF-I-treated group, a significant increase in bone area was recognized at three days after administration. Maor *et al*.[5] found no increase in bone area in IGF-I-treated neonatal condyles *in vitro*. This difference may be due to the difference in the experimental conditions (*in vitro* vs. *in vivo*).

Spencer *et al*.[10] reported that when IGF-I was intra-arterially administered to the growth plate of the proximal tibia in 6-week- and 3-month-old rats, there were almost no histological changes in the growth plate in 6-week-old rats. They concluded that the administration of an overdose of IGF-I to the growth plate in rapidly growing rats dose not further facilitate changes compared to its effects in mature rats. In our study, the local administration of IGF-I to 3-week-old rat condyle caused no change in the amount of endochondral bone growth or the thickness of the cartilaginous layer. This might be because condylar growth at this age was so rapid that the administration of excessive IGF-I could not induce further growth.

In 3-week-old rat condyle, although no significant differences in the width of the cartilaginous layer or the amount of endochondral bone growth were seen in comparison with the control group, increased bone tissue was seen at the primary subchondral cancellous bone layer. Suzuki[24] reported that, in the normal condyle, the percentage of bone area in the subchondral cancellous bone layer increased with aging. In this study, although an increase in bone area in the subchondral cancellous bone layer was observed at three days after the local administration of IGF-I in 3-week-old rat condyle, the bone area gradually decreased and returned to the same level as in the control group by seven days after administration. These results suggest that the effect of IGF-I seen in this study may not have been due to an acceleration of maturation, but rather was a transient effect of IGF-I on the cells involved in bone remodeling.

In 12-week-old rat condyle, the local administration of IGF-I caused an increase in the thickness of cartilaginous layer and a decrease in bone area in the subchondral cancellous bone layer. These findings are opposite the histological changes seen in normal maturation in the condyle, since the thickness of the cartilaginous zone decreased and the bone area in the subchondral cancellous bone layer increased with maturation in the normal condyle. The histological changes in this study suggest that the condylar cartilage may be stopped from converting to articular cartilage and has characteristics of growth cartilage upon resuming endochondral bone formation, although further detailed histo-quantitative evaluation is necessary.

In conclusion, this study suggests that the local administration of IGF-I may make it possible for the mandibular condyle to continue growing even after normal growth is complete. However, it may be difficult to further accelerate mandibular condylar growth in the period of rapid growth.

Acknowledgements

This study was supported in part by a Giant-in-Aid for Scientific Research (10307052) from the Ministry of Education, Science, Sports and Culture of Japan. We are grateful to the Research Facilities for Laboratory Animal Science, Tokyo Medical and Dental University Faculty of Medicine.

References

1. Isaksson OGP, Ohlsson C, Bengtsson BA, et al. GH and bone -Experimental and clinical studies. Endocr J 2000;47(Suppl):S9-S16.
2. Ohlsson C, Vidal O. Effects of growth hormone and insulin-like growth factors on human osteoblasts. Eur J Clin Invest 1998;28:184-86.
3. Scheven BAA, Hamilton N. Longitudinal bone growth in vitro: effects of insulin- like growth factor I and growth hormone. Acta Endocrinol (Copenh) 1991;124:602-7.
4. Schlechter NL, Russell SM, Spencer EM, et al. Evidence suggesting that the direct growth-promoting effect of growth hormone on cartilage in vivo is mediated by local production of

somatomedin. Proc Natl Acad Sci USA 1986;83:7932-4.
5. Maor G, Hochberg Z, Silbermann M. Insulin-like growth factor I accelerates proliferation and differentiation of cartilage progenitor cells in cultures of neonatal mandibular condyles. Acta Endocrinol (Copenh) 1993;128:56-64.
6. Maor G, Laron Z, Eshet R, et al. The early postnatal development of the murine mandibular condyle is regulated by endogenous insulin-like growth factor-I. J Endocrinol 1993;137:21-6.
7. Maor G, Hochberg Z, Silbermann M. Growth hormone stimulate the growth of mouse neonatal condylar cartilage in vitro. Acta Endocrinol (Copenh) 1989;120:526-32.
8. Visnapuu V, Peltomäki T, Rönning O, et al. Growth hormone and insulin-like growth factor I receptors in the temporomandibular joint of the rat. J Dent Res 2001;80:1903-7.
9. Hunzilker EB, Wanger J, Zapf J. Differential effects of insuline-like growth factor I and growth hormone on developmental stages of rat growth plate chondrocytes in vivo. J Clin Invest 1994;93:1078-86.
10. Spencer EM, Liu CC, Si CC, et al. In vivo actions of insulin-like growth factor-I (IGF-I) on bone formation and resorption in rats. Bone 1991;12:21-6.
11. Isaksson OGP, Londahl A, Nilsson A, et al. Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocrin Reviews 1987;8:426-38.
12. Isgaard J, Nilsson A, Lindahl A, et al. Effects of local administration of GH and IGF-1 on longitudinal bone growth in rats. Am J Physiol 1986;250(Endocrinol Metab 13):E367-72.
13. Russell SM, Spencer EM. Local injections of human or rat growth hormone or of purified human somatomedine-C stimulate unilateral tibial epiphyseal growth in hypersectomized rats. Endocrinology 1985;116:2563-7.
14. Schoenle E, Zapf J, Humbel RE, et al. Insuline-like growth factor I stimulate growth on hypophysectomized rats. Nature 1982;296:252-3.
15. Noguchi K. Effects of extrinsic forces on the mandibular condyle of the young rat - Observations using ^{3}H-thymidine autoradiography-. Kokubyo Gakkai zasshi 1970;37:222-41.
16. Enlow DH. Handbook of Facial Growth, ed 2. Philadelphia: W.B.Saunders, 1982.
17. Scott JH. Dentofacial Development and Growth. London: Pergamon Press, 1967;65-173.
18. Sarnat BG. Facial and neurocranial growth after removal of the mandibular condyle in the macaca rhesus monkey. Am J Surg 1957;94:19-30.
19. Robinson IB, Sarnat BG. Growth pattern of the pig mandible. Am J Anat 1955;96:37-64.
20. Symons NBB. Studies on the growth and form of the mandible. Dent Rec 1951;71:41-53.
21. Weinmann JP, Socher D. Bone and bones. St. Louis: The C.B. Mosby Co, 1947;55-122.
22. Yamamoto M. Effects of human growth hormone on mandibular condyle growth of the rat. J Fukuoka Dent Coll 1990;17:145-60.
23. Isaksson OGP, Jansson JO, Gause IAM. Growth hormone stimulate longitudinal bone growth directly. Science 1982;216:1237-9.
24. Suzuki S. Histomorphometric study on growing condyle of rat. Bull Tokyo Med Dent Univ 1986;33:23-34.
25. Durkin JF, Heeley JD, Irving JT. The cartilage of the mandibular condyle. Oral Sci Rev 1973;2:29-99.