INCREASING OF GLYCOSAMINOGLYCAN AND HYDROXYPROLINE CONTENT IN RAT CARTILAGE MANDIBLES AFTER INSULIN LIKE GROWTH FACTOR–I INDUCTION

(PENINGKATAN GLIKOSAMINOGLIKAN DAN HIDROKSIPROLIN PADA TULANG RAWAN MANDIBULA TIKUS SETELAH INDUKSI INSULIN LIKE GROWTH FACTOR-I)

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Abstract

The cellular effects of Insulin Like Growth Factor-I (IGF-I) are mediated by binding to cell IGF-I receptors, which are found mainly on cells of mesenchymal origin, such of fibroblast, chondrocytes, and osteoblast. The study suggests that IGF-I may regulate chondrocytes proliferation, differentiation and extracellular matrix deposition. The purpose of this study was to quantify a synthesis of hydroxyproline HYP and glycosaminoglycan (GAG) by primary chondrocyte culture with IGF-I at 0, 5, 25, and 50 ng/ml concentration. Chondrocyte proliferation was assessed with collagen synthesis and measured by using hydroxyproline (HYP) assay and glycosaminoglycan GAG synthesis was determined with dimethylmethylene blue dye binding assay for 10 days. Primary chondrocytes was cultured for 10 days with 0, 5, 25 and 25 ng/ml IGF-I. The results showed that, IGF-I was a potent in increasing GAG synthesis and also effective in promoting collagen HYP synthesis. At the 50 ng/ml concentration, IGF-I was upregultators of GAG synthesis, producing 6 times more GAG than control. Collagen content was promoted by IGF-I at its lower concentration, with level 3 times higher than control. In conclusion, IGF-I at doses ranging from 5 to 50 ng/ml for ten days has proved the induction GAG and HYP synthesis in 10 days primary chondrocyte culture of secondary cartilage rat condyles.

Key words: primary chondrocyte culture, glycosaminoglycan, hydroxyproline

Abstrak

Efek selular Insulin Like Growth Factor-I (IGF-I) diperantarai melalui ikatan IGF-I reseptor, yang terutama ditemukan pada sel asal yaitu sel mesenkim, seperti fibroblast, kondroblast, dan osteoblast. Penelitian ini menunjukkan IGF-I dapat mengatur proliferasi kondrosit, diferensiasi dan deposit ekstraselular matiks. Tujuan penelitian kuantitatif ini adalah mengukur sintesis kolagen dengan kultur primer kondrosit dan IGF-I pada konsentrasi 0, 5, 25, and 50 ng/ml. Proliferasi kondrosit dihitung dengan sintesis kolagen dan diukur menggunakan sintesis hydroxyproline HYP assay and glycosaminoglycan GAG, yang ditentukan dengan dimethylmethylene blue dye binding assay pada hari ke-10. Kultur primer kondrosit dikultur selama 10 hari dengan IGF-I pada konsentrasi 0, 5, 25, and 50 ng/ml. Hasil penelitian menunjukkan bahwa IGF-I potensial dalam meningkatkan sintesis GAG dan sintesis kolagen HYP. Pada konsentrasi 50 ng/ml, IGF-I meningkatkan sintesis GAG, produksi GAG enam kali lebih tinggi dari kontrol. Jumlah kolagen dipromosikan melalui IGF-I pada konsentrasi yang rendah, dengan tingkat tiga kali lebih tinggi dari kontrol. Sebagai kesimpulan, IG-I dengan dosis dari 5 sampai 50 ng/ml selama 10 hari terbukti dapat menginduksi sintesis GAG dan HYP dalam 10 hari kultur primer kondrosit dari kartilago sekunder kondili mandibula tikus.

Kata kunci: kultur kondrosit primer, glikosaminoglikan, hidroksiprolin

INTRODUCTION

The theories of craniofacial growth and development have often been discussed. Some theories have been proposed, of which some define the mandibular condyle as an important growth centre.[1]

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Cartilage of the mandibular condyle is considered as secondary cartilage, while the epiphyseal growth plate, synchondrosis and the cartilage of femoral head are all considered as primary cartilage.[2] Secondary cartilage contains of a pre-chondroblast. Cell proliferation in vivo as well as in organ culture occurs only among pre-chondroblasts. As this mesenchymal-like cells later differentiates into chondroblasts producing cartilaginous matrix, they lose their ability to divide.

IGF-I was detected in the mandibular condyle of growing rats, and localization was shown to be dependent on the stage of development.[2] Moreover, Visnapuu *et al,*[3] and Ardani and Sjafei[4] found differences in the distribution of IGF-I receptors in the mandibular condyle of young rats. This finding indicates that growth factors might indeed differentially regulate secondary cartilage.

IGF-I is known to be an important regulator of chondrogenesis and also stimulated proliferation in the study.[5] It has been proved that IGF-I stimulated cell proliferation and differentiation in primary chondrocyte culture of rat mandibular condyle.[6]

IGF-I has potent mitogenic and metabolic effects, both when it added exogenously and when it secreted by their cultured cells.[2] It has also shown that IGF-I increased GAG synthesis in mandibular condyle cartilage. Other studies have also found that IGF-I stimulates the production of proteoglycans in chicken growth plate chondrocytes, in bovine chondrocytes and growth plate condrocyte. Also, in mandibular condylar cartilage from neonatal rats increased GAG synthesis. GAG synthesis appears to play a key role in the processes of cellular proliferation and differentiation that occur during chondrogenesis in the limb bud.[7]

The purpose of this study was to quantify glycosaminoglycan and hydroxyprolene synthesis by primary chondrocyte culture from 4-day-old Wistar rats for 10 days incubation by IGF-I at 0, 5, 25, and 50 ng/ml concentration.

MATERIALS AND METHODS

Breeding groups of rats were kept under normal laboratory conditions and were fed standart rat chow and water ad libitum. The experiment was approved by Board for Annimal Experiments of Faculty of Veteriner-University of Airlangga (Ethical clearance No. 012-KE).

Subjects used 20 secondary cartilage of 14-day-old Wistar rats (*Rattus Noevegicus*). The rats were decapitated 20 mandibular condyles and dissected under dissecting microscope observation. Throughout this procedure, the tissue was bathed in MEM supplemented with gentamycin, thiomerzal and vitamin C. After removing the adhering soft tissue and muscles, the mandibular condyles were chopped with scalpel. The cell explants splinted into 16 well in 2 ml culture medium per well (in 46-well culture). Every 200 ml culture medium consisted of 1.63 gr MEM; 80 μl gentamycin; 0.02 gr thiomerzal; 20 μl vitamin C; 10% Newborn Bovine Serum (Biological Industries) and IGF-I (Labvision) in different dose 0, 5, 25, and 50 ng/ml. The primary cell was cultured at 37 ^{0}C in a humidified incubator in an atmosphere of 5% CO2 in air. The culture medium was changed every two days.

Aliquots of the digest were also analysed for total suphated GAG with a spectrophotometric asaay using dimethyllene blue. The Hydroxyproline (HYP) content was determined after alkaline hydrolysis. The HTP and GAG contents were as total amount per culture and expressed as percentages of the control cultures.

The cells were collected and digested by pepsin overnight for analyzing total hydroxyproline content. The hydroxyproline content measured for amount of collagen, was determined after alkaline hydrolysis and read using spectrophotometer. Hydroxyproline content was determined as total amount per aliquots of chondrocyte primary culture of mandibular rat condyle (as described in Biocolor protocol). Statistical analysis used ANOVA test followed by Turkey test.

RESULTS

The GAG synthesis of the primary chondrocytes culture od secondary cartilege mandiblar rat were analysed after 10 days in culture with different concentrations of IGF-1 (Table 1). The mean value of GAG synthesis in the control was 0.82, while the treatment culture of GAG synthesis was increased. The stimulation of GAG synthesis was significant at all concentrations of IGF-I ($p<0.05$) (Table 1). The results expressed concentration denoted showed significant difference ($p<0.05$) between the control and treated primary chondrocyte culture of secondary cartilage rat condyle. A denotes showed a significant difference between control and treated primary chondrocyte culture of secondary cartilage rat condyles (Table 1).

GAG and HYP synthesis of the primary chondrocyte culture of secondary cartilage rat condyle analyzed after 10 days culture with 0, 5, 25, 50 ng/ml IGF-I (Figure 1 and 2). In 50 ng/ml IGF-I increased the GAG synthesis six times higher than in control growth ($p<0.05$). With 50 ng/ml IGF-I, increased in HYP synthesis 3 times higher than con-

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trol growth and was also significant ($p<0.05$). IGF-I was found to increase significantly GAG and HYP in a dose-related manner (Table 2).

Table 1. Mean of glycosamonogen and hydroxyproline content in primary tissue culture of rat condyle for 10 days IGF-1 stimulation

IGF-I (ng/ml)	N	Mean GAG	Mean HYP	p
0	7	0.82 ± 0.043	5.1 ± 0.29	0.00
5	7	9.05 ± 0.13	14.97 ± 0.19	0.00
25	7	15.18 ± 0.25	20.31 ± 0.43	0.00
50	7	67.66 ± 0.49	35.09 ± 0.38	0.00

Table 2. Correlations of Glycosamonogen and Hydroxyproline content in primary tissue culture of rat condyle for 10 days IGF-I stimulation

IGF-I (ng/ml)	N	corelation (r)	p
GAG	28	0.94	0.000
HYG	28	0.95	0.000

DISCUSSION

The results presented in this study clearly showed that GAG and HYP synthesis significantly increased in the primary chondrocyte culture of secondary cartilage rat condyle. These increases may reflect the chondrocyte proliferation and differentiation.[8]

A classification of carbohydrates of particular interest is represented by proteoglycans (PGs) and glycosaminoglycans (GAGs). These polysaccharides are pivotal in dermal matrix structure that embed and sustain collagen fibers network. These considerations raised the concept of a new class of potential in gun altered matrix structure through stimulating GAG and PG synthesis.[9]

IGF-I is known to be an important regulator of chondrogenesis. Hamerman et al.[9] has found that cartilage-specific proteoglycans are formed in chondrocyte. Growth factor cartilage-derived from growth factor (CDGF) and fibroblast growth factor (FGF) have different effects on GAG synthesis.[7]

After 10 days, HYP content of the primary chonrocyte culture of secondary cartilage rat condyles was measured without IGF-I (as control), with 5, 25, 50 ng/ml (Figure 2). HYP content of cultures increased in control growth ($p<0.05$) (Table 2).

Secondary cartilage of 4-day-old rats was composed of various zones and showed a thick layer of hypertrophied chondrocytes as described by Delatte et al.[8] The cartilage persisted for only a limited time as the most of chondrocytes become hypertrophied. This transformation could result from the lack of function and growth factors are necessary for its regulation.[1, 3, 9] In the mandibular condyles, the only proliferation cell population was that in the chondroprogenitor layer. The prechondroblasts lose their ability to divide when they differentiate in to matrix-synthesizing chondroblasts from chondrocytes. Cartilage of mandibular condyle, growth is appositional. During development, the secondary cartilage is involved in growth processes at early stage. This type of cartilage, growth determined by cell proliferation, matrix synthesis and cell hypertrophy. The mandibular condyle, cell division occur on superficial.[1-3]

The study about IGF-I receptor analyses was performed to determine the effect on IGF-I on the secondary cartilage. The results showed that IGF-I stimulated growth and significantly different compared to the control group.[4]

In the mandibular condyle of the young rat, mitotic activity occurs not only in the undifferentiated cells but also in the differentiated chondroblasts of the proliferative layer. At a later age, the chondroblasts are no longer dividing in the condylar cartilage.[3]

In vitro examination of the mandibular condyle of neonatal mice indicates that the production of IGF-I is paralleled with the distribution of IGF-I receptors, both being located in the chondroprogenitor and chondroblast layers. These results are in line with the present in vivo observations, although the younger rats also displayed some sporadic reactions in the lower hypertrophic layer of the condylar cartilage, i.e., the active layer would appear to decrease with age. Nevertheless, it is proposed that IGF-I is a potent chondrogenic agent which, consequently, constitutes a fundamental factor in the tissue-separating morphogenic capacities of various cartilage-containing structures, including the mandibular condyle.

The chondrogenic potency of IGF-I is also evident from previous experimental studies, since administration of IGF-I to young rats has been found to be followed by accelerated chondrogenesis in the condylar cartilage.[14] Effects of growth factors and glucosamine on porcine mandibular condylar cartilage cells and hyaline cartilage cells for tissue engineering applications.[5]

IGF-I was found to increase GAG and HYP in a dose-related manner. Statistical analysis revealed a strong association between GAG and HYP synthesis and the IGF-I dose increased. It was considered to be necessary to relate the depth of the IGF-I-labeled cell layer to the overall depth of the condylar head, since, during the present evaluation period, considerable reduction is known to occur in its amount of cartilage.[7, 10]

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The enhanced cellular proliferation, along with the increased synthesis of proteoglycans, resulted in a substantially larger mass of tissue in the organ culture system. The nature of the IGF-I stimulative effect was further studied through the use of a tissue culture system whereby a separated chondroprogenitor zone is cultured under conditions which favorits development at first into cartilage and then into bone. Using this culture system, we could show that IGF-I induces merely the de novo chondrogenesis proces. This was reflected in the appearance of relatively large amounts of cartilage specific antigens such as cartilage proteoglycans and chondrocalcin. This result indicated that IGF-I is a strong chondrogenetic agent.

In conclusion, on the basis of IGF-I at doses ranging from 5 to 50 ng/ml for ten days. IGF-I was found to increase significantly GAG and HYP in a dose-related manner. It can be postulated that the effects of the agents on the growth of the secondary cartilage mandibular rat condyle. IGF-I is produced locally in the condylar cartilage. It is also proved in this study that GAG and HYP sintesis were positive in the culture without IGF-I treated.

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