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EFFECT OF DIFFERENCE MECHANICAL FORCE INDUCTION TO OSTEOCLAST AND OSTEOBLAST HEAT SHOCK PROTEIN 25 EXPRESSION

(EFEK PERBEDAAN INDUKSI KEKUATAN MEKANIS TERHADAP EKSPRESI HSP25 OSTEOKLAS DAN OSTEOBLAS)

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Abstract

The usage of orthodontic appliance is to correct malocclusion involves alveolar bone remodeling. This process is stimulated by mechanical force which obtained from the activation orthodontic appliance. The force was used to depress teeth and the tissues surrounding it. This stress will become a signal to activate heat shock response yielding HSP synthesis. HSP25 plays a part in osteoclastic bone resorption and osteoblastic new bone forming. The aim of this study was to explore the change of osteoclast and osteoblast HSP25 expression in the different mechanical force induction at pressure and strain area. The subject was guinea pig (Cavia sp), divided into 2 groups. Control group did not use orthodontic appliance and treatment group was given mechanical force as 90 g, 120 g, and 150 g. Time usage of orthodontic appliance was 5 and 10 days. Osteoclast and osteoblast HSP25 were measured by counting cells after being conducted by immunohistochemistry method. The result showed that osteoclast HSP25 expression at pressure area on all groups had increased value compared to strain area and was different statistically. On the other hand, osteoblast HSP25 expression at strain area had increased value was compared to at pressure area and especially in group of 120 g and 10 days had difference statistically. In conclusion, different mechanical force induction could influence osteoclast and osteoblast HSP25 expression. HSP25 may represent one of the factors that influences osteoclast and osteoblast cellular activity and coupling process between osteoclast and osteoblast.

Key words: osteoclast, osteoblast, mechanical force

Abstrak

Penggunaan alat ortodonsia untuk mengkoreksi maloklusi tergantung pada proses remodeling tulang alveolar. Proses ini distimulasi oleh gaya mekanis yang didapat dari aktivasi alat ortodonsia. Gaya digunakan untuk menekan gigi dan jaringan disekitarnya. Tekanan ini akan menghasilkan sinyal untuk aktivasi *heat shock response* termasuk sintesis HSP. HSP25 memainkan salah satu peran pada resorpsi tulang oleh osteoklas dan pembentukan tulang baru oleh osteoblast. Tujuan penelitian ini adalah untuk mencari perubahan ekspresi HSP25 osteoklas dan osteoblas yang diinduksi gaya mekanis yang berbeda pada daerah tekanan dan regangan. Subyek penelitian menggunakan marmut (Cavia sp), dibagi menjadi 2 kelompok. Kelompok kontrol tidak menggunakan alat ortodonsia dan kelompok perlakuan diberi gaya mekanis sebesar 90 g, 120 g dan 150 g. Waktu penggunaan alat ortodonsia adalah 5 hari dan 10 hari. HSP25 osteoklas dan osteoblas diukur melalui penghitungan sel-sel setelah dilakukan metode imunihistokimia. Ekspresi HSP25 osteoklas pada daerah tekanan di semua kelompok mempunyai nilai yang meningkat dibanding area regangan dan berbeda bermakna secara statistik. Sebagai kesimpulan, ekspresi HSP25 osteoblas meningkat nilainya pada daerah regangan jika dibandingkan dengan daerah tekanan khususnya pada kelompok 120 g dan waktu 10 hari berbeda bermakna secara statistik. HSP25 memungkinkan sebagai salah satu faktor yang mempengaruhi aktivitas seluler osteoklas dan osteoblas dan proses *coupling* antara osteoklas dan osteoblas.

Kata kunci: osteoklas, osteoblas, gaya mekanis

INTRODUCTION

The usage of orthodontic appliance is to correct malocclusion entangle alveolar bone remodeling process. The remodeling process can be stimulated by using mechanical force obtained from activation of orthodontic appliance to depress tooth and periodontal tissue including gingiva, cementum, periodontal ligament and alveolar bone.[1] Activation is conducted based on adequate encumbering in tooth. In practice, the factor plays a part in encumbering is the ability and the skill of dentist who takes care patient. Mechanical force is often given inadequately.

The mechanical force given will cause the area around the tooth split in two areas that is pressure and strain. At pressure area, mechanical force will stimulate osteoclast to resorp alveolar bone together with the local factor (hormone or the other chemical mediator). Inadequate force causes a very small alveolar bone resorption or does not happen, while excessive force can activate osteoclast to abundant resorption (undermining resorption).[2]

Mechanical force used of orthodontic appliance will give stress at periodontal tissue. This mechanical stress will become signal activity heat shock response, causing expression of heat shock genes. Expression of this gene is generated by activation of stress-induced transcription factors that is heat shock factor (HSF) and binding with *heat shock promoter element* (HSE) had characteristic as pentanucleotide of 5'nGAAn-3' motive. The effect of activation of HSF, cell will synthesize a protein molecule as heat shock protein (HSP).[3]

HSP represents protein molecule of intracellular which becomes a signal to some biological cell activeties. Group of HSP which plays an important role in human bone remodeling is HSP27, where at rodent animal has homologue that is HSP25. Some re-searches show a role of HSP25 at osteoclast and osteoblast.

At osteoblast, HSP27 syntesis influences was used to regulate osteoblast differentiation which was inducted by Prostaglandin (PG) $F_{2\alpha}$, endothelin-1, sphingosine 1-phosphate, basic fibroblast growth factor (bFGF) and PGD_2.[4] Kawamura explained that HSP27 accumulation in osteoblast differentitation can be inducted by endothelin-1 via p38 MAP kinase activation pathway. Hatakeyama, et al. Explained that HSP27 can regulate osteoblast function to stress condition (heat) and play its function coorporate with estrogen.[5]

At osteoclast, HSP27 plays in bone resorption in order to release calcium[6], and Kawamura, et al. explained that HSP27 regulates osteoclast on bone resorption function is inducted by endothelin-1, and this shows that alveolar bone remodeling is a coupling process where osteoclast and osteoblas correlated one another. Pursuant to the matter above shows that the level of mechanical force can influence expression of osteoclast and osteoblast HSP25.

The aim of this study was to explore the change of osteoclast and osteoblast HSP25 expression in the different mechanical force induction at pressure and strain area.

MATERIALS AND METHODS

The animal that used in this research was Guinea pig (*Sp. Cavia*) and fullfil the following criterias, healthy physic and did not have disparity, male, aged 3-5 months weight 350-550 g and also given the same food. Animals were acclimatized during one week for adaptation with food and place before given treatment.

The animals were divided into 2 groups, control and treatment groups. Control group did not use orthodontic appliance. Treatment group was given mechanical force related at previous research with little modification.[8,9] as 90 g (inadequate force). 120 g (adequate force), and 150 g (excessive force). The length time of using orthodontic appliance was 5 and 10 days.

Orthodontic appliance used stainless steel wire 0.12 inch (class one, US) for simple cantilever, angle between cantilever arm 60°, length of cantilever arm 1 cm and coil diameter 1.5 mm. This design produced mechanical force 4 oz/120 g, and was obtained other force by changing distance between cantilever arm.

The animals were anaesthetized by using ketamin with a dosage of 44 mg/kg subcutaneous. Tooth preparation was done at mesial part of interdental lower incisor about 5 mm distance from insical with round shape. Appliance attached and stabilized with Glass Ionomer Fuji type of IX.

The specimen used to immunohistochemical method was alveolar bone in mesial part of incisor (strain area representation) and distal part (pressure area representation). Specimen conducted decalcifycation by giving citrate acid 5% for 5 days. And immunohistochemistry procedure was done according to factory guidance. Detection of HSP25 used primary antibody of Rabbit Anti-Hsp25 Polyclonal Antibody (Stressgene Bioreagent) and secondary antibody of *Anti rabbit Ig G Biotin-labelled*. Expression of osteoclast and osteoblast HSP25 counted to use bright field microscope (Olympus CX 31), magnification of 1000 X at 5 slides from each

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restating. Expression of HSP25 was shown in percentage, obtained from cell expressing HSP25 compared to the cell population and converted in percentage.

To know the change of osteoclast and osteoblast HSP25 expression in the different mechanical force induction at pressure and strain area between treatment groups were analyzed by one way ANOVA using computer program.

RESULTS

The result showed that expression of HSP25 osteoclast at pressure area at all of treatment force and time treatments had larger value and statistical difference ($p>0.05$) if compared to strain area. The pressure area represented activity of osteoclast in bone resorption of alveolar. This trend was also experienced by expression of osteoblast HSP25 at strain area, that had bigger value than pressure area. Especially in treatment time group of 5 days and treatment force of 120 g had statistical difference ($p<0.05$). Strain area represented activity of osteoblast in forming new bone (Table 1).

Table 1. Average of HSP25 expression of osteoclast and osteoblast at pressure and strain área base on treatment time and force

Treatment time (day)	Treatment force (g)	Osteoclast HSP25 expression		Osteoblast HSP25 expression	
		Pressure area	Strain area	Pressure area	Strain area
Control	-	29,17 ±19,43		7,97 ± 2,65	
5	90	32,41 ± 6,84	16,67 ± 12,50	6,27 ±2,74	5,17 ± 1,67
	120	39,51 ± 10,06	19,75 ± 17,15	8,67 ± 0,91	9,77 ± 0,89
	150	38,18 ± 9,02	22,23 ± 15,75	7,05 ± 2,75	7,66 ± 3,88
10	90	33,44 ± 12,83	6,94 ± 6,48	12,93 ± 2,86	12,23 ± 2,57
	120	35,65 ± 10,85	14,81 ± 14,30	8,87 ± 4,40	14,42 ± 6,29
	150	42,33 ± 7,51	14,29 ± 11,90	9,84 ± 5,35	13,02 ± 7,32

DISCUSSION

The application of different mechanical force in this research aimed to observe osteoclast and osteoblast HSP25 expression at pressure and strain area. Osteoclast and osteoblast were chosen because both cells have important role at alveolar bone remodeling bone process.[2]

Mechanical force induced alveolar bone remodeling responded to the first time by osteocyte, where osteocyte will secrete some mediators used to regulate the process of osteoblast differentiation. Osteoblast besides functioned to form new bone also secreted some important mediators for the process of osteoclast differentiation and function in bone resorption. Osteoclast besides did its own function, this cell can also regulate osteoblast function through mediator which was secreted. Chen et al. explained that in mechanical stimulation, osteoblast for the first time will response with mediated by prostaglandin production. This mechanical stimulation responded by activation of phospholipase A2 that released by acid arachinoid and increased prostaglandin production. Increasing of prostaglandin level can activate adenyl cyclase (cAMP) so that cAMP level increased, and it will also trigger proliferation process. This cell would release the other mediators to do new bone forming and trigger osteoclast forming and function in bone resorption. Joldersma et al. explained that prostaglandin required in signal pathway of new bone forming process, where bone cell especially osteocyte and osteoblast will increase PG production when mechanical force was applicated.[9] PG was produced via releasing of arachidonic acid from phospholipid staying in membrane which mediated by phospholipase A2. This process was followed with conversion of arachidonic acid become PGG2 and subsequently altered to PGH2 via mediated prostaglandin B/H synthase enzyme (cyclooxygenase= COX). PGH2 isomered become active prostanoid biologically, as PGE2, PGI2 and of $PGF_{2\alpha}$.

Kohno et al.[10] also explained that mechanical stimulation which was adequate value might quicken process of alveolar bone remodeling. Where on day 10 at strain area, osteoblast was possible to express vascular endothecial growth factor (VEGF) was used to angiogenesis process and related to forming of new bone. On the other hand, this mediator also improved the number and function of osteoclast in bone resorption. This idea was supported by Azuma et al. explained that VEGF represented candidate as mechanotransducer for the activation of cellular process hereinafter in response with mechanical change.[11]

Aikawa et al. and Steven et al. also explained that mechanical stress plays important role at some tissue remodeling and morphogenesis.[12] The change of cell morphology happened restructuring of cytoskeleton component and cell attaching modulation, causing specific signal intracellular from cell attaching receptor and induced special gene expression. Mechanical stimulation will activate MAPK in order to repeat gene expression and improve protein synthesize. MAPK consists of *extracellular signal–regulated kinases* (ERKs), *c-Jun NH2-terminal kinase* (JNK), and p38 MAPK. Azuma et al. also

explained that p38 MAPK activetion result ranscriptional factor activation and HSP27 synthesize with end result in the form of cell transformation and migration.[11] On the other hand, according to Steven et al. explained that cytoskeletal remodeling which was inducted by mechanical occurrence would cause native HSP27 will phosphorilate as induction of MAPK p38 activation.[13]

The process of new alveolar bone forming at strain area requires osteoclast HSP25 regulation so that osteoblast does its function. The increasing of HSP25 osteoclast expression is probable related to process of Hepatocyte factor growth (HGF) synthesis and secretion. HGF was needed to regulate by autocrine at osteoclast and by parakrin to osteoblast. HGF did both cells by HGF receptor activation that followed by increasing of Ca^{2+} intracellular concentration via activation of pp6Oc-src kinase.[14] HGF induced transformation, chemotactily migration stimulation and DNA replication at osteoclast and improvement of osteoblast DNA synthesis.

Katagiri et al. explained that the process of osteoclast differentiation and function was regulated by osteoblast through chemical mediator release.[15] For example, releasing of Macrophage-Colony stimulating factor (M-CSF) by osteoblast influenced osteoclast early differentiation. Baron and Krishnan et al. also explained that osteoclastic resorption process and oteoblastic new bone forming cooperation through coupling process, including chemical mediator and other molecule which influenced process.[16,17] Ruimerman et al. also explained that mechanical induction in bone produced corporation between osteoclast that did resorption process and osteoblast that did new bone bone process, where alveolar bone forming process was done osteoblast would trigger and improve indirect resorption process was done by osteoclast.[18]

It can be concluded that different mechanical force induction can influence expression of osteoclast and osteoblast HSP25. HSP25 may represent one of the factors influences cellular osteoclast activity and osteoblast individually and also coupling process between both cells.

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