The Expression Of Heat Shock Protein (Hsp) 25 At Compression And Tension Area DuringAlveolar Bone Remodeling

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Abstract

The activation of orthodontic appliance will generate mechanical force. This force is used to depress tooth and the tissues surrounding it then stimulates alveolar bone remodeling. Alveolar bone remodeling is divided into compression and tension area. This stress becomes a signal to activate heat shock response yielding heat shock protein (HSP) synthesis, especiallyhsp25isessential foralveolarboneremodelingprocessitself. The aim of this study was to compare hsp25 expression in compression and tension areas. This study using guinea pigs mandibular first insicivus that given a mechanical force to see hsp25 expression in both areas. The HSP25 expressionwas measured by counting this protein after being conducted by immunohystochemistry method. It is analysed statistically with one way Anova with level of significant p = 0,05.

Key words: Heat shock protein (HSP)25, compression area, tension area, alveolar bone remodeling

Ekspresi Heat Shock Protein (Hsp) 25 Pada DaerahTekanan Dan Regangan Selama RemodelingTulang alveolar

Abstrak

Aktivasi alat Ortodonsia akan menghasilkan gaya mekanis. Gaya ini digunakan untuk menekan gigi dan jaringan disekitar gigi yang nantinya menstimulasi remodelling tulang alveolar.Remodeling tulang alveolar dibagi menjadi daerah tekanan dan regangan. Stress ini menjadi sinyal untuk mengaktivasi heat shock response yang mengakibatkan sintesis heat shock protein (HSP), khususnyahsp25 yang penting untuk proses remodeling tulang alveolar. Tujuan penelitian ini adalah untuk membandingkan ekspresi hsp25 pada daerah tekanan dan regangan.Penelitian ini menggunakan gigi insisivus pertama rahang bawah marmut (guinea pigs) yang diberi gaya mekanis untuk melihat ekspresi hsp25 di kedua daerah tersebut. Ekspresi hsp25 di analisa dengan menghitung penampakkan protein ini setelah dilakukan metode imunihistokimia.Data dianalisa secara statistic dengan uji beda one way Anova dengan tingkat kemaknaan p = 0,05.

Kata Kunci : Heat shock protein (HSP)25, daerah tekanan, daerah regangan, remodeling tulang alveolar

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Introduction

Remodeling process can be stimulated using mechanical force obtained from activation of orthodontic appliance to depress tooth and periodontal tissue.(1) Activation is conducted based on adequate encumbering at tooth.

The mechanical force given will cause the area around the tooth split in 2 areas i.e compression and tension areas. At compression area, mechanical force will stimulate osteoclast to resorp alveolar bone together with the local factor (hormone or the others chemical mediator). Inadequate force causes alveolar bone resorption very small or does not happen, while excessive force can activate osteoclast to abundant resorption (undermining resorption).(2)

Mechanical force used in orthodontic appliance will give stress at periodontal tissue hit by mechanical force. This mechanical stress will become signal activity of heat shock response, causing expression of heat shock genes. Expression of this gene is generated by activation of stress-induced transcription factors i.e heat shock factor (HSF) and binding with *heat shock promoter element* (HSE) having characteristic as pentanucleotide of 5'nGAAn-3' motif. 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 activities. Group of HSP which plays an important role in human bone remodeling is HSP27, where at rodent animal has homologue that is HSP25. The aim of this research is to observe HSP25 expression at compression and tension areas during alveolar bone remodeling.

Material And Method

The animal used in this research is Guinea pigs (*Sp. Cavia*) with following criteria, physically-healthy and do not have disparity, male, 3 – 5 months, 350-550g and also supplied with the same food. Animals were acclimatized during one week for adaptation with the food and the place before given treatment.

Animals were divided into 2 groups, control and treatment groups. Control group is without the usage of orthodontic appliance. The treatment group given by mechanical force of 120 g. Time of orthodontic appliance use is 5 and 10 days.

Orthodontic appliance to be used in the animals and mechanical force value related to the previous research with little modification.(4,5) Orthodontic appliance is stainless steel wire 0,12 inch (class one, US) with simple cantilever form, angle between cantilever arm 60°, length of cantilever arm 1 cm and coil diameter 1,5 mm. This design produces mechanical force as 4 oz / 120 g.(6)

The animal anaesthetized using ketamine with a dosage of 44 mg/kg subcutaneous. Tooth preparation was done at mesial part of interdental lower insisivus that was 5 mm distance from insical with round shape. The appliance was

attached and stabilized with Glass Ionomer Fuji type of IX.

The specimen used in immunohistochemical method is alveolar bone in mesial part of insicivus(tension area representation) and distal part (compression area representation). Specimen conducted decalcification (provided with citrate acid 5% during 5 days). immunohistochemistry procedure was subsequently conducted according to factory guidance. Detect of HSP25 use 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 restating. HSP25 expression counted from brown spot appearance at tension and compression areas.

Data resulted from enumeration and measurement between treatment groups were analyzed by one way ANOVA and then LSD post hoc test SPSS ver. 13 program with significance level (p) < 0,05.

Result

The counting of HSP25 expression aim at observing osteoclast and osteoblast HSP25 expression when given different mechanical force and time of mechanical force. Result of HSP25 expression counting is presented at Table 1.

Table 1. The HSP25 expression at compression and tension area

Treatment group	area	day	HSP25 Expression
Group I			5.72 ± 1.65
Group II	Compression	5	8.72 ± 3.89
Group III	Compression	10	5.17 ± 1.25
Group IV	Tension	5	7.11 ± 3.10
Group V	Tension	10	9.50 ± 3.02

The table shows that the lowest HSP25 expression is in the group III, whereas the highest is in the group V. Based on the area, in the group of compression area, group II (5 days) possesses greater value compared to the group III (10 days) and significantly different (p<0,05). While in the tension area, group V (10 days) possesses higher value compared to group IV (5 days) and significantly different (p<0,05).

Discussion

The value of HSP25 expression in treatment group is higher compared to control group. It shows that HSP25 was expressed when mechanical force was loaded on tooth continued to periodontal tissue and induced heat shock gene.

Based on the area, group II (5 days) possesses greater expression value and is significantly different

compared to group III (10 days). It shows that at group II, HSP25 seems to play important role in regulating osteoclast cells to initiate resorption process, while group III shows reduced cellular expression related to osteoclast performance that begins to reduce in resoption process.

At osteoclast, HSP27 plays in bone resorption in order to release calcium [7,8], and Kawamura, *et al.*[9] explain 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 osteoblast have correlated one another.

On the other hand, in the tension area, group V (10 days) possesses greater expression value and significantly different compared to group IV (5 days). It demonstrates that in the group V, HSP25 seems to play important role in regulating osteoblast cells to initiate new alveolar bone formation, while the group IV shows lower HSP25 expression related to osteoblast performance that has not started its functions and activities in forming new alveolar bone.

At osteoblast, HSP27 synthesis influences is used to regulate osteoblast differentiation which is inducted by Prostaglandin(PG) $F_{2\alpha}$, endothelin-1, sphingosine 1-phosphate, basic fibroblast growth factor (bFGF) and PGD_2.[10] Kawamura, *et al.* [9] explains that HSP27 acumulation in osteoblast differentitation can be inducted by endothelin-1 via p38 MAP kinase activation pathway. Hatakeyama, *et al.*[11] explain that HSP27 can regulate osteoblast function to stress condition (heat) and play its function cooperate with estrogen.

The result of this research demonstrates that HSP25 expression increases when the function of and activity of bone cells both osteoclast in compression area and osteoblast in tension area increase as well.

Conlussion

HSP25 plays important role in regulating osteoclast function in the compression area and osteoblast in tension area in the process alveolar bone remodeling activated by mechanical force.

References

1. Proffit W R.. Contemporary orthodontics. Toronto: The CV Mosby Company, 1986: 230-4.
2. Mulyani. Biomekanis pergerakan gigi. Jakarta: Penerbit Widya Medika, 1994: 37-40.
3. Morimoto, R. I., 1998. Regulation of The Heat Shock Transcriptional Respone: Cross Talk Between a family of Heat Shock Factor, Molecular Chaperones, and Negative Regulators, *Gene & Development* 12:3788-3796.
4. Sintesa S M, Soemarko L S dan Hermawan I. Pengaruh hambatan prostaglandin pada pemberian aspirin, diklofenak dan paracetamol terhadap pergerakan gigi, jumlah sel osteoklas dan osteoblas tulang alveolus gigi rahang atas akibat pemakaian alat ortodontik (eksperimen hewan coba). Tesis.

Malang: PS S2 Biomedik, Program Pascasarjana Universitas Brawijaya, 2003.

5. Yani S M, Soemarko L S, Setyohadi R. Peranan radikal bebas superoksid (O_2^o) terhadap peningkatan resorpsi tulang alveolar akibat penggunaan alat ortodontik. Tesis. Malang: PS S2 Biomedik, Program Pascasarjana Universitas Brawijaya, 2003.
6. Tipler, Paul A. Fisika untuk sains dan tehnik, Jilid 1. Alih Bahasa. Lea Prasetio, Rahmad W. Adi. Jakarta: Penerbit Erlangga, 1998: 102.
7. Gaston, J. S. H., 2002. Heat shock proteins and innate immunity. *Clinical and Experimental Immunology*, 127:1–3.
8. Nair SP, Meghji S, Reddi K, Poole S, Miller AD, Henderson B., 1999. Molecular chaperones stimulate bone resorption. (Abstracts)*Calcif Tissue Int.* 1999 Mar; 64(3):214-8.
9. Kawamura, H., Takanobu Otsuka, Hiroyuki Matsuno, Masayuki Niwa, Nobuo, Matsui, Kanefusa Kato, Toshihiko Uematsu and Osamu Kozawa, 1999. Endothelin-1 stimulates heat shock protein 27 induction in osteoblasts: involvement of p38 MAP kinase, *Am J Physiol Endocrinol Metab* 277:1046-1054.
10. Tokuda, H. O Kozawa, M. Niwa, H. Matsuno1, K. Kato, T. Uematsu, 2002. Mechanism of Prostaglandin E2-stimulated Heat Shock Protein 27 Induction in Osteoblast-like MC3T3-E1 Cells, *Journal of Endocrinology 2002* ,172: 271–2
11. Hatakeyama, D., O. Ozawa, M. Niwa, H. Matsuno, K. Kato, N. Tatematsu, T. Shibata and T. Uematsu, 2001. Inhibition by adenylyl cyclase-cAMP system of ET-1-induced HSP27 in osteoblasts, *Am J Physiol Endocrinol Metab* 281: E1260–E1266.