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The Expression of Collagen Type-I in the Tension Area of Orthodontic Tooth Movement with Adjuvant of Hyperbaric Oxygen Therapy

Arya Brahmanta[1], Soetjipto[2], IB Narmada[1]

[1]Departemen of Orthodontic Faculty of Dentistry, Universitas Airlangga Surabaya, Indonesia.
[2]Departemen Biochemistry Faculty of Medicine, Universitas Airlangga Surabaya, Indonesia.
[1]Departement of Orthodontic Hang Tuah University Surabaya, Arif Rahman Hakim 150, Surabaya, Indonesia.

Abstract : Objective: To verify the effect of Hyperbaric Oxygen Therapy (HBO) towards the expression of type I collagen on the tension area of orthodontic tooth movementin remodeling process of periodontal ligament.
Meaterial and Methods: Twenty four Caviacobaya males were divided into three groups. (X1) negative control. (X2) orthodontic groups, 0.0474 kN force was applied to the maxillary incisors, (X3) orthodontic force 0.0474 kNwith addition of 7 session HBO 2.4 ATA 90 minutes. The expression of type I collagen was measured by using immunohistochemistry technique and analyzed by ANOVA followed by LSD test($p< 0.05$).
Results: After 14 days of treatment, imunohistochemistry analysis revealed that addition of 7 session HBO 2.4 ATA 90 minutes increased the expression of type 1 collagen on the tension area. The mean of groups (X1), (X2), and (X3), were (8.87 ± 2.232), (10.00 ± 2.138), and (15.37± 1.685). ANOVA test showed a significant difference in all the groups ($p<0.05$).
Conclusions: Hyperbaric Oxygen therapy in seventh days effectively increasing the expression of type I collagen in the tension area of the periodontal ligament, this approach might be a feasible treatment strategy to accelerate orthodontic tooth movement.
Keywords: Orthodontic tooth movement, HyperbaricOxygen Therapy, Type I collagen.

Introduction

The orthodontic tooth movement is a process in which the application of the force causes absorption in the compressed side and formation of new bone in the tensile side. The applied pressure within the periodontal ligament which is produced by the orthodontic forces is considered as the main factor for tooth movement. [1]

A law in orthodontics is that a tooth can be moved through the alveolar bone when an appropriate orthodontic force is applied. This is based on the principle that a change in mechanical loading of a biological system results in strain, which subsequently leads to cellular responses aiming at adaptation of the system to the changed conditions. As a result of this principle, remodelling of the periodontal ligament (PDL) and the alveolar bone around a tooth takes place during orthodontic force application.[2,3]

Bone remodeling is characterized as a cyclic and lengthy process. It is currently accepted that not only

this dynamics is triggered by a biological process, but also biochemical, electrical, and mechanical stimuli are key factors for the maintenance of bone tissue. [4]

Contemporary orthodontics is not about biomechanical procedures only, but it is also including periodontal physiology too, since the mutual influence between the periodontal and teeth exceeds the physiological aspects to the mechanism of the treatment. [1]

The Orthodontic force gives rise to the histological findings referred to as zones of tension and compression area. In the compression area, orthodontic force induced deformations of the periodontal ligament, resulting in disturbances of circulation, decreasing the blood perfusion of the tissue. This condition is accompanied by hypoxia, which is known to either affect cell proliferation or induce apoptosis, depending on the oxygen gradient.[3] On tension area, electron microscopy has shown a significant reduction of collagen fibre diameter. It is believed thatthe extension of fibres during the remodeling process results in the elongation of these fibers, allowing for tooth movement.[5]

Orthodontic forces are known to have various effects on the alveolar process, such as cell deformation, inflammation, and circulatory disturbances. Each of these conditions affecting cell diferentiation, cell repair, and cellmigration, is driven by numerous molecular and inflammatory mediators. Fibroblasts, osteoblasts, osteocytes, osteoclasts, odontoblasts, cementoblasts, chondrocytes and immune cells are the major cell types involved in the remodeling process. Fibroblasts are the major group of cells found in the Periodontal Ligament (PDL). PDL contains primarily the type I and type III collagen fibers and the type I is the dominant collagen.[6,7]

Collagen type I is the resulting expression of the highest collagen (90%) of osteoblasts, where as osteocalcin is a non-specific collagen produced only by osteoblasts. Both collagens have essential role in the formaton of the matrix in the regeneration process.[8]

Hyperbaric oxygen therapy (HBO) is the inhalation of 100% oxygen inside a hyperbaric chamber that is pressurized to greater than 1 atmosphere absolute (ATA; 760 mm Hg). HBO is typically administered at 1 to 3 ATA. While the duration of an HBO session is typically 90 to 120 minutes.[9]

Reactive oxygen species (ROS) are produced as a natural byproduct/intermediates in biological processes in body by the normal oxygen metabolism. The role of free radical reactions in disease pathology iswell established and is known to be involved in many acute and chronic disorders in human beings. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells. [10]High oxygen on circulation will lead ephitelialization of cells also to reduce and kill bacterial which cause an infection. Addition of oxygen in wound healing in encouraged the formation of new connective tissue, also encourage the formation of Fibroblast Growth Factor-2 mediated presence of macrophages and platelets. [11]

Specific growth factors involved in wound healing during HBO treatment are basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and transforming growth factor (TGF-β1). HBO daily treatment at 2 ATA selectively stimulates fibroblast proliferation after 7 days.[12]HBO has been shown to induce vascular endothelial growth factor (VEGF) expression in human umbilical vein endothelial cells and increase nitric oxide levels in perivascular tissues via stimulation of nitric oxide synthase. The beneficial effects of HBO in treating ischemia related wounds may be mediated by stimulating collagen synthesis, cell proliferation, and promoting angiogenesis.[13]Transforming growth factor beta (TGF-β) is a cytokine synthesized in bone tissue and platelets that stimulates the proliferation of osteoblast precursor cells, promotes collagen synthesis, increases the number of cells that express the osteoblast, increases osteoclast apoptosis, and activates endothelial cells for angiogenesis.[14]

Angiogenesis is a complex process involving extensive interplay between cell soluble factors and extra cellular matrix (ECM) components. The angiogenesis involve different sequential steps including: The release of proteases from activated endothelial cells, degradation of the basement membrane surrounding the existing vessel, migration of the endothelial cells into the interstitial space, endothelial cell proliferation, lumen formation, generation of new basement membrane with the recruitment of pericytes, fusion of the newly formed vessels, Initiation of blood flow.[15]

The aims of this study was to verify the effect of Hyperbaric Oxygen Therapy 2,4 ATA 90 minutes

towards the expression of type I collagen on the tension of orthodontic tooth movement.

Experimental

Ethical permission was obtained from the Faculty of Dentistry, Airlangga University Ethics Committee of Experimental Animal Use and the Research Scientific Committee (151/KKEPK.FKG/X/2014).

A number of 24 male Guinea pig (*caviacobaya*) 3-4 month old is divided into three groups, the age of three to four months with a average weight 300-400 gram, were given orthodontic tooth movement on the maxilla with orthodontic force by elastic separator.

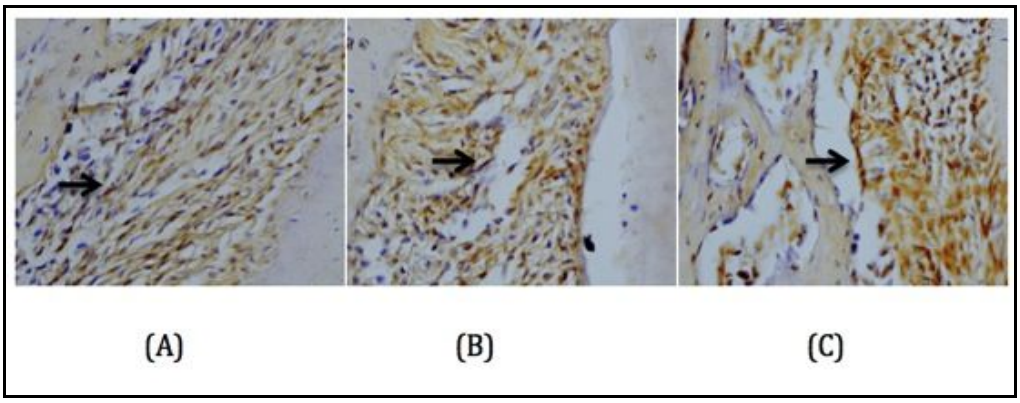

Figure 1. The expression of type I collagen on the tension of orthodontic tooth movement. A). Control groups X1, B). Ortho groups X2, C). Ortho + HBO groups X3

After adaptation of animals for 1 week, the animal were randomized into 3 groups, as follows: Control groups (X1) is a groups without further treatment, Ortho groups (X2) animals subjected to orthodontic force only for 14 days and HBO groups (X 3) animals subjected to orthodontic force day 1-14 then treated with therapy of daily HBO 2,4 ATA 90 minutes, totally 7 sessions on day 8-14. HBO sessions were conducted using a veterinary hyperbaric chamber model

After 2 weeks of treatment, caviacobayaeuthanasia was induced by deep anesthesia with xylazine then the maxilla of each animal, was surgically removed and placed in 10% buffered formalin. After this period, the decalcification process was begun in ethylenediaminetetraacetic acid (EDTA). After 30 days, the pieces were embedded in paraffin. Paraffin blocks were cut with a microtome and sections were stained immunohistochemistry (IHC) to determine expression of collagen. The slides were observed under a light microscope at 400x magnification.

The data obtained were calculated for statistical analysis. Analysis of variance (ANOVA) and multiple comparisons by the LSD test were used. For all the results a significance level of $p<0.05$ was used. All statistical analyses were performed with SPSS *program software*, version 20.

Results

The results of research data analyzed in descriptive statistics obtained seen in table 1, where the results of the count of type Icollagen expression in each group shows differences in average value of .The table shows increase type I collagen expression with the provision of HBO therapy in the tension area of orthodontic tooth movement who helped remodeling ligaments periodontal. Based on the results of the test LSD show there were differences increased expression type I collagen meaningful ($p< 0.05$) on all groups except group X1 with groups of X2.

Table 1 - Descriptive statistic, average ± standard deviation of the expression of collagen type 1 according to groups

Groups	Mean ± SD
X1	8,87 ± 2,232
X2	10,00 ± 2,138
X3	15,37 ± 1,685

Table 2.*Post-Hoc LSD* the expression of collagen type 1 according to groups *(*p < 0.05)*

Groups	**X1**	**X2**	**X3**
X1	-	.281	.000*
X2	.281	-	.000*
X3	.000*	.000*	-

Discussion

The results obtained from this experimental showed that average number of type I collagen was highest in the treatment group with the provision of therapy HBO 2.4 ATA for 7 days. Periodontal ligaments had an important role in the process of the movement of the teeth because of its ability in responding to the mechanical force that cause the remodeling of bone. Khouw and Goldhaber investigated vascular changes in the PDL after 1,3 and 7 days by applying tipping forces onto the teeth of rhesus monkeys and German shepherd dogs. After 24 hours of force application, vessels on the tension side of the root were widened, whereas blood vessels on the pressure side showed partial or complete occlusion.[5,16]

By compressing the PDL, orthodontic force was induced cell deformation in alveolar tissues, stimulating mechanosensitive ion channels and receptors in the cell membrane. Periodontal cells seem to react to mechanical stimulation by upregulating cellular mediators. Fibroblasts, osteoblasts, osteocytes, osteoclasts, odontoblasts, cementoblasts, chondrocytes and immune cells are the major cell types involved in the remodeling process. [5, 7] At the apposition side, the principal fibres are stretched and remodelling of the PDL takes place. New bone is formed by the activated osteoblasts that first produce new ECM (extra cellular matrix) and then mineralize this in a unidirectional manner. The principal fibres of the PDL will also be entrapped in the newly formed bone as Sharpey'sfibres. In the meantime, new PDL matrix is formed to maintain the width of the PDL and the attachment of the tooth to the alveolar bone. This new PDL contains thick principal fibres mainly type I collagen for attachment of the tooth to the bone. Fibroblasts are the major group of cells found in the PDL . The PDL contains primarily the type I and type III collagen fibers and the type1 is the dominant collagen. The principal and oxytalan fibers are the predominant elastic fibers, which provide elasticity to the ligament during the tension related force on the ligament. [7,17,18]

Collagen fiber is primarily synthesized by fibroblast as procollagen protein,which is secreted and further processed to be a collagen fiber in the extracellular matrix. Collagen degradation is mainly regulated by MMPs, while collagen synthesis is mediated by both transcriptional (gene/mRNA level) and post translational (protein level) processes.[19]

Oxygen is essential in the process of collagen synthesis. It controls the hydroxylation of proline and lysine during the collagen synthesis process. In the absence of oxygen, collagen synthesis cannot take place. Previous studies showed that ATA between 2.0 to 2.5 increases the amount of oxygen dissolved into the plasma, thereby increasing the oxygen tension was found to be beneficial for bone formation if applied for 90-120 minutes per day. HBO has been shown to influence cellular proliferation positively as the process is oxygen dependent. [20,21] HBO may promote angiogenesis, which is vital for bone healing. HBO therapy resulted in increased VEGF expression. Vascular endothelial growth factor (VEGF) is one of the key factors that stimulates angiogenesis.[22]HBO is beneficial because it stimulates the growth of new blood vessels and results in a substantial increase in tissue oxygenation that can arrest certain types of infections, enhance oxygenation, fibroblast proliferation, collagen synthesis, ephithelialization and neovascularization.[13,23]Neovascularization is

the growth of the vascular system which plays a major role in both health and diseases.[15]

Collagen synthesis is considered an essential element in wound healing. HBO has been shown to influence this process positively with an oxygen partial pressure of 150mmHg being the optimum level. According to Broussard et al, fibroblasts cannot proliferate in tissue cultures in the absence of oxygen. [20,21,22]Fibroblasts are considered to be mechanoresponsive, in that the mechanical signals transmitted from the ECM via integrin receptors influence their morphology, cytoskeletal organization, proliferation, differentiation, and gene expression. After orthodontic force application, the density of cells expressing positive signals for type I collagen mRNA is greater in the PDL in tension sites. [23, 24]

HBO has been shown to promote the expression of vascular endothelial growth factor (VEGF) known to stimulate angiogenesis, while depositions extracellular matrix, collagen synthesis propagated by growth factors and cytokines namely PDGF (*platelet-devived*), FGF(*fibroblasts growth factor*), TGF (*transforming growth factor*) and IL-1, IL-4 (*interleukin*) that produced by leukocytes and lymphocytes when collagen synthesis. [20, 25,26,27]The role of oxygen in collagen synthesis is well established. Oxygen is essential in the hydroxylation of proline and lysine during collagen synthesis. In the absence of oxygen, collagen synthesis cannot take place.[22]A partial pressure of 150 mmHg was shown to be the optimum level for the synthesis. HBOT has shown a positive effect on the cross linking of collagen. [20] The provision of therapy HBO 2.4 ATA increased expression of type I collagen in the tension area of orthodontic tooth movement, can help remodeling ligaments periodontal in orthodontic treatment because 80 % collagen contained in anperiodontal ligament is type I collagen .

Conclusions

As the conclusions HBO therapy 2.4 ATA 90 minutes for 7 days influence in increasing expression type I collagen in the tension area against remodeling on ligaments periodontal of orthodontic tooth movement.

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