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The increasing of fibroblast growth factor 2, osteocalcin, and osteoblast due to the induction of the combination of Aloe vera and 2% xenograft concelous bovine

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ABSTRACT

Background: To make a successfull denture prominent ridge is needed, preservation on tooth extraction socket is needed in order to prevent alveol bone resorption caused by revocation trauma. An innovative modification of the material empirically suspected to be able reduce inflammation caused by the revocation trauma is a combination of Aloe vera and xenograft concelous bovine (XCB) and Aloe vera is a biogenic stimulator and accelerating the growth of alveolar ridge bone after tooth extraction. Purpose: The research was aimed to determine of the increasing alveol bone formation by inducing the combination of Aloe vera and 2% xenograft concelous bovine. Methods: To address the problems, the combination of Aloe vera and xenograft concelous bovine was induced into the tooth extraction sockets of Cavia cabayas which devided on 8 groups. Groups control, filled with XCB, Aloe vera and Aloe vera and XCB combination, at 7 days and 30 days after extraction. Afterwards, immunohistochemical examination was conducted to examine the expressions of FGF-2 and osteocalcin, as the product of the growth of osteoblasts. Results: There were significantly increases expression of FGF-2 and osteocalcin on group which filled with XCB, Aloe vera and combined Aloe vera and XCB. Conclusion: It may be concluded that the induction of the combination of Aloe vera and xenograft concelous bovine into the tooth sockets can enhance the growth expressions of FGF-2 and osteocalcin as the product of osteoblasts, thus, the growth of alveolar bone was increased.

Key words: Fibroblast growth factor 2, osteocalcin, osteoblast, alveolar bone, combination of Aloe vera and graft

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INTRODUCTION

The success of denture manufacture requires supporting anatomical condition of oral cavity, especially prominent ridge. To achieve the good condition of ridge, after tooth extraction, the ridge must be prevented from resorption first. Some users of fixed denture and partially removable denture feel uncomfortable with the dentures due to sustained resorption of residual ridge after tooth extraction, and this situation may cause structural changes in jaw bone. Level of residual ridge resorption in each person, however, is not the same, so we need to conduct the prevention of residual ridge resorption.  

After tooth extraction, the alveolar ridge usually decreases about 50%, and this condition occurs in the period of 6-12 months. The decreasing even occurs several times due to residual ridge resorption that physiologically occurs after tooth extraction. Residual ridge resorption is a biomechanical disease caused by multi-factors, such as anatomic, metabolic, and mechanical determinants. After tooth extraction, there will usually be inflammation as rapid reaction in the area of extraction, and the sockets of the extraction will be filled with blood clot. Later, the epithelial tissue will be proliferated and migrated in one week, and the tissue will fix the sockets of the extraction quickly.

The use of bovine graft to improve either damaged bone or augmentation is actually often conducted in the field of general surgery and oral surgery. However, the use of bovine graft still can not give satisfactory results. Therefore, we need an innovative material, biogenic stimulator, to stimulate graft that can accelerate bone growth.

Aloe vera has been considered as biogenic stimulator and hormonal activator in healing wound. Aloe vera extract can be used to prevent scar tissue during incision, and when Aloe vera gel is used after surgery, the incision will heal quickly. Significant changes of the amount of collagen of the control and treatment groups indicate how Aloe vera can stimulate fibroblast cell growth and wound healing process. Thus, this research was aimed to prove whether the induction of the combination of Aloe vera and 2% xenograft concelous bovine (XCB) into tooth extraction sockets can increase the expression of FGF2 and osteocalcin, which then may cause the increasing of alveolar bone growth.

MATERIALS AND METHODS

This research was an experimental research with randomized post-test control group design with the combination of Aloe vera and 2% XCB. The combination of Aloe vera and 2% XCB can indicate that each active substance contained is about 2%. XCB is the product of Bank of tissue of Dr. Soetomo Hospital Surabaya. Other materials used in this research consist of Aloe vera extracts, sterile distilled water, XCB, absolute alcohol, 70% alcohol, anti-FGF2 monoclonal antibodies, anti osteokalcin monoclonal antibodies, and reagents. Those materials were used for immunohistochemical examination, and reagents used for staining was Hematoxilin-eosin (HE). Instruments used in this research, moreover, were a set of tools for both immunohistochemical examination and preparation manufacture, such as micropipette, tip (yellow, white, blue), light microscopy, object glass, and cover glass.

Animals used in this research, furthermore, were healthy and active male Cavia cobaya (marmots) with 300 -350 grams of weight and in the age of 3 to 3.5 months. Those animals were then divided into 8 groups. Next, lower right incisor extraction was then conducted in all those groups. Firstly, Cavia cobaya were taken from their care center, and then anaesthetized with intra venous ketamine 0.2 cc/300g BM. The tooth extraction process was conducted by using special pliers (needle holder). After the tooth extraction, the sockets were filled with materials as much as 0.1 cc, and then stitched. The materials were loaded as follows: group I: the sockets of tooth extraction were filled with polyetilene glycol (PEG) as the control group, and then examined after 7 days; group II: the sockets of tooth extraction were filled with PEG (as the control group), and then examined after 30 days; group III: the sockets of tooth extraction were filled with xenograft concelous bovine (XCB) and PEG, and then examined after 7 days; group IV: the sockets of tooth extraction were filled with XCB and PEG, and then examined after 30 days; group V: the sockets of tooth extraction were filled with Aloe vera and PEG, and then examined after 7 days; group VI: the sockets of tooth extraction were filled with Aloe vera and PEG, and then examined after 30 days; group VII: the sockets of tooth extraction were filled with Aloe vera, XCB, and PEG, and then examined after 7 days; and group VIII: the sockets of tooth extraction were filled with Aloe vera, XCB, and PEG, and then examined after 30 days.

After 7 days and 30 days, after being decalcified with 2% nitric acid, the jaws of those tested animals were cut to make preparations of block paraffin. Afterward, slides were made for staining with imunnohistochemistry and hematoxilin eosin (HE). Next, the calculation was conducted on FGF-2, osteocalcin, and immunohistochemical staining by using microscopic examination, and then the amount of osteoblasts and osteoclasts was calculated on HE staining.

The tested animals were taken care at Biochemical Laboratory of Medical Faculty, Universitas Airlangga, while the manufacture of tissue preparation was conducted at Histology Laboratory and Pathology Anatomi Laboratory of Dr. Soetomo Hospital. Moreover, the manufacture of Aloe vera extract was conducted at Physical Chemistry Laboratory of Pharmacy Faculty, Universitas Airlangga, while the manufacture of Aloe vera freeze dried was conducted at Biology Laboratory of Science and Technology Faculty, Universitas Airlangga. And, immunohistochemical examination was conducted at Immunohistochemistry Laboratory, LPT Tropical Disease Center (TDC) of Universitas Airlangga, and at Biomedical Laboratory of...
Universitas Brawijaya. The calculation results were then recorded and tabulated. Afterwards, the result data were analyzed by using ANOVA, and then multiple comparison of the data of those four groups was conducted.

RESULTS

The increasing of the expressions of FGF-2 and osteocalcin in the control group and in the treatment groups induced with XCB, Aloe vera, and Aloe vera on day 7 and day 30 can be seen in Figure 1. Based on Figure 1, it can be indicated that the expressions of FGF2 and osteocalcin in those three treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB were higher than those in the control group. However, the highest expressions of FGF-2 and osteocalcin were found in the group induced with Aloe vera + XCB on day 30. The expression of FGF-2 in those four groups can be seen in Figure 2.

Based on the Figures 2, it is known that the expressions of FGF-2 in the treatment groups induced with XCB (2b), Aloe vera (2c), and Aloe vera and XCB (2d), increased more as pointed by black arrows than those in the control group (2a). Meanwhile, the expressions of osteocalcin on day 30 of immunohistochemical examination can be seen in Figures 3a, b, c, d.

Based on Figures 3, it is known that the expressions of osteocalcin in the treatment groups induced with XCB (3b), Aloe vera (3c), and Aloe vera + XCB (3d) increased more as pointed by black arrows than those in the control group (3a).

Based on ANOVA analysis, moreover, it is also known that there was significant difference of the expressions of FGF-2, p<0.05, between the control group and the treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB. Similarly, there was also significant difference of the expressions of osteocalcin, p<0.05, between the control group and the treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB.

Figure 1. The average expressions of FGF-2 and osteocalcin in the control group, in the treatment groups induced with XCB+PEG, Aloe vera + PEG, and Aloe vera + XCB + PEG on day 7 and on day 30.

Aloe vera (3c), and Aloe vera + XCB (3d) increased more as pointed by black arrows than those in the control group (3a). Based on ANOVA analysis, moreover, it is also known that there was significant difference of the expressions of FGF-2, p<0.05, between the control group and the treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB. Similarly, there was also significant difference of the expressions of osteocalcin, p<0.05, between the control group and the treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB.

Figure 2. a) The expression of FGF-2 on day 30 in the control group; b) The expression of FGF-2 on day 30 in the group induced with XCB; c) The expression of FGF-2 on day 30 in the group induced with Aloe vera; d) The expression of FGF-2 on day 30 in the group induced with Aloe vera dan XCB. Black arrows indicate the expressions of FGF-2.
Furthermore, based on Tukey HSD analysis, there was significant difference of the expressions of FGF-2, p<0.05, between the control group and the groups induced with XCB, Aloe vera, and Aloe vera + XCB. There was also significant difference of the expression of osteocalcin between the control group and the treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB. However, there was no significant difference between the group induced with Aloe vera and the group induced with XCB.

Next, the expressions of FGF-2 and osteocalcin on day 7 and on day 30 were analyzed by using t-test. The results then showed that there was significant difference of the expressions of FGF-2 and osteocalcin, t<0.05, started from day 7 to day 30. Finally, how the increasing of the expressions of FGF-2 and osteocalcin stimulated the growth of osteoblast can be seen in Figure 4.

Based on Figure 4, it is known that the growth of osteoblasts in the control group and in the treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB increased from day 7 to day 30 (see red bar). And, the highest growth of osteoblasts occurred in the group induced with Aloe vera + XCB on day 30.

**DISCUSSION**

Based on Figure 1, it is known that that the expressions of FGF2 and osteocalcin in the control group and in the treatment groups induced with XCB, Aloe vera, and Aloe vera + XCB increased started from day 7 to day 30 of the examination. The highest FGF-2 expression and osteocalcin were found in the group induced with Aloe vera + XCB started from day 7 to day 30 of the examination. It indicates that Aloe vera + xenograft concelous bovine can stimulate the growth of alveolar bone in the socket of tooth extraction. Healing process with graft induction can prevent alveolar bone from resorption prior to the formation of new alveolar bone. Thus, graft material used must be biocompatible, so it can inhibit bone resorption and stimulate osteogenesis.
This opinion is also supported by some experts stating that graft induced into tooth extraction sockets can cause osteoinduction, stimulating bone growth.\textsuperscript{8-11}

The increasing of osteocalcin expression can also be caused by osteocalcin mostly produced by osteoblasts. There are actually collagen and non-collagen proteins in osteoblasts, which usually synthesize non-collagen protein in bone matrix, namely osteocalcin and osteonectin as much as 40–50\% in bone.\textsuperscript{12} Fibroblast growth factor 2, furthermore, is an important modulator for both the growth of bone as well as the differentiation, expression and regulation of osteoblast.\textsuperscript{11} In addition, FGF-2 is also an important signaling molecule for the regulation of bone development stages, thus, if FGF-2 increases, the amount of osteoblasts will also increase.\textsuperscript{13,14}

The expressions of FGF-2, osteocalcin, and osteoblast in the group induced with \textit{Aloe vera} were higher than those in the group induced with XCB. It indicates that \textit{Aloe vera} is an anti-inflammatory, antibacterial, anti-virus because it contains not only anthraquinones components, namely aloin, aloe emodin, and barbaloin, but also pure mannan carbohydrate, acetyl mannan glucomannan, as well as alkaline phosphatase and bradikinase enzymes. There are also healing hormones, auxin, gibberellin, and saponins, as well as proteins in \textit{Aloe vera} that can enhance tissue and bone healing process so that \textit{Aloe vera} can reduce inflammation caused by tooth extraction trauma, then can induce the healing process of the wound, and can finally stimulate osteoblast, as consequence, the growth of new bone will increase.\textsuperscript{4,15-22} This increasing process is also stimulated by \textit{Aloeride} as polysaccharides derived from \textit{Aloe vera} that has a high molecular weight and strong immunostimulator activity.\textsuperscript{23}

Thus, the group induced with \textit{Aloe vera} and XCB had the highest expressions of FGF2, osteocalcin, osteoblasts, and fibroblasts started from day 7 to day 30 of examination. It indicates that Aloe vera and XCB in tooth extraction cases can accelerate wound healing process, can prevent inflammation, and then can induce new bone growth. It is because when Aloe vera is combined with XCB which is osteoinduction, it will accelerate healing process and new bone growth.\textsuperscript{2,8,9,10}

The increasing of osteoblast will not occur continuously, but at a certain time it will decrease. In a previous study, it is known that the growth of osteoblasts in tooth extraction socket filled with \textit{Aloe vera} and XCB with 0.5\% concentration of the active substance increased on day 30, but on histologic examination it is known that the growth of osteoblasts decreased on day 60.\textsuperscript{24} On day 12 after the tooth extraction, the formation of new bone occurred along the socket wall as well as in trabecular spaces around the tooth extraction area, and trabecule bone was also woven at the periphery socket, osteoprogenitor cells, preosteoblasts, and osteoblasts. Periodontal ligament also moved into the middle of the socket, but not attached to the socket wall, and then got apoptosis. Collagen density, as a result, increased, and then was gradually replaced by bone. After this phase of bone resorption, freezing, granulation, and collagenase phases of the healing process can be bypassed, and then the socket of tooth extraction may regenerate.\textsuperscript{7} Finally, it may be concluded that the induction of the combination \textit{Aloe vera} and 2\% xenograft concelous bovine into the socket of tooth extraction can increase the expressions of FGF2 and osteocalcin, so the growth of the alveolar bone can increase.

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