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Carbonated hydroxyapatite inflammation's responses on local rabbits: study of neutrophils's cell count, macrophages, and edema volumes on mandible

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ABSTRACT

Objective: The aims of this study was to find out the response of tissue inflammation implanted CHA than standard HA through the amount of neutrophil, macrophage, and edema volumes.

Methods. Sixty local rabbits divided into 3 groups that contain 20 rabbits. Each group divided into 5 sub groups that observed in day-1, 3, 5, 7, and 14. On first day, all rabbits were anesthesized and incised to create cavity in the mandible. CHA or HA powder as much as 0,05 g were put into it. Then it was sutured using silk thread. On the designated day, the mandibles of 4 rabbits from each sub group were decapitated. The mandibles were fixed with 10% formalin in PBS pH 7,4. *Von Ebner* method for decalcification were used followed by *hematoxylin eosin* staining to examine neutrophil and macrophage.

Results: The number of neutrophil, macrophage, and edema volumes in CHA implantation were lower than HA. There were not any significant difference (p>0,05) between CHA, HA, and without CHA or HA.

Conclusion: There are no differences inflammation respons in CHA implantation and HA.

1. Introduction

Bone destruction, one of common complication, happened in dentistry especially oral surgery. Bone damage was defined as a cleft of the bone which is needs to be filled with new bone. Moreover, changes in the alveolar bone influenced the teeth [1]. Several materials and techniques such as bone graft, guided tissue regeneration (GTR), or growth factor had some role in periodontal cell growth and development. It already used in regenerative therapy for bone destruction [2].

Bone graft had several types. Based on the donor, bone graft divided into autograft (from the patient), allograft (from other people), xenograft (from animal), and alloplasgraft (synthetic)[3,4]. Autograft, allograft and xenograft had several limitations such as donor morbidity, rejection, and disease transmission.

Hydroxyapatite (HA) was unique material in the bone ingredient which has chemical formula ($Ca_{10}(PO_4)_6(OH)_2$)[5]. HA material had been used in dentistry and had high biocompatibility with human body. The main compound of HA was carbonate moreover the main structure of human bone was carbonate apatite (CHA)[6].

Biological activity of CHA was better than HA. CHA had higher solubility, lower crystallization, smaller crystal morphology, and higher chemical reactivity than HA [7,8,9]. CHA had higher in vivo solubility. It increased calcium and phosphate concentration which is important for new bone development[10.11.12] furthermore CHA can be used in bone remodeling.

Every material which is put into the body must had good biocompatibility. Generally, biocompatibility was measured from cytotoxicity, systemic response, allergic reaction and carcinogenetic [13]. One of biocompatibility test that must be done was inflammation response test.

Implantation of material into the body induced several events such as damage, acute inflammation, chronic inflammation, granulated tissue formation, antigen reaction, and fibrosys

[14]. Several processes induced inflammation reaction, such as surgery process or from the material characteristic. This research was done to find the differences of inflammation response of animal mandible after implantation of HA or CHA based on several parameters such as neutrophil cell count, macrophage cell count, and edema volume.

2. Materials and Methods

This research was to examine the inflammation response from bone substitution material in mandible after implantation CHA, HA and without CHA or HA with duration of implantation was 1, 3, 5, 7, and 14 days. Inflammation response was measured from neutrophil, macrophage cell count, and edema volume of mandible. Moreover, the sample of this research was 60 locally male rabbits. Every group consist of 20 rabbits and it divided into 5 sub-groups which was consists of 4 rabbits.

CHA material was collected from integrated research laboratory, Faculty of Dentistry Universitas Gadjah Mada. CHA and HA powder was weighed and sterilized by UV sterilizer for 20 minutes. The animal was anesthesized and the fur was cut in mandible area and then the thickness of the mandible was measured. The mandible was incisized until periosteum and created the cavity by bone drill (round and fissure drill) for 3mm x 3mm x 3mm and irrigate with NaCl to clean residue of drilling process. As many 0,05g CHA and HA powder mixed with physiologic NaCl was

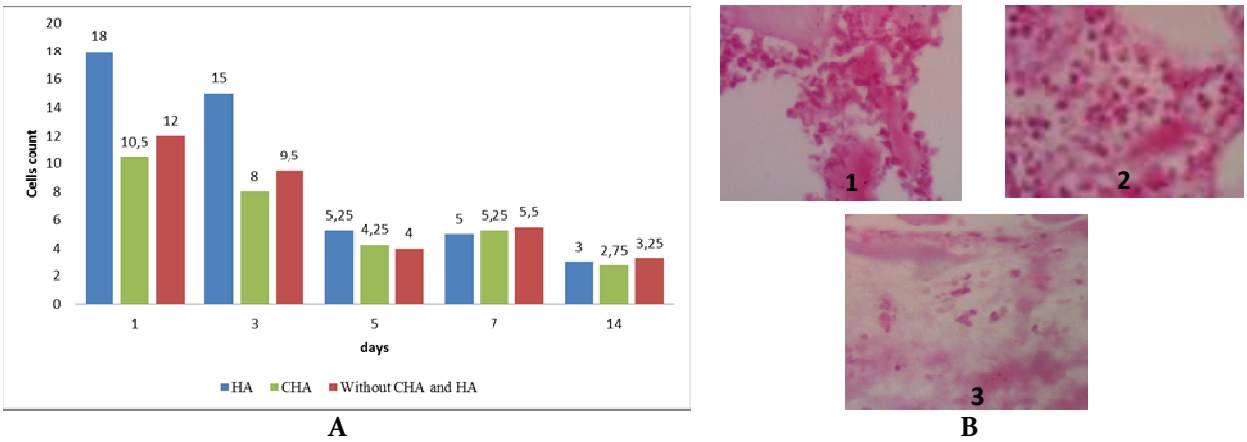


Figure 1. A) Average number of neutrophil cell on rabbit mandible based on implantation time, B) Histological image of neutrophil cell on implantation of CHA (1), HA (2), and without CHA and HA (3).

inserted into the cavity. The tissue surrounding the cavity was put it back and sewed by silk tread. At 1, 3, 5, 7 and 14 days after implantation, the mandible of the animal was measured the edema, then decapitated and took the mandible. The mandible was fixed in PBS formalin 10% at pH 7,4 and was decalsificated by Von Ebner methods and also Hematoxylin Eosin (HE) coloring methods to see the neutrophil and macrophage cells.

The number of neutrophil and macrophage was analyzed at 10 areas by using light microscope with 400x dilatation. The data was analyzed by two ways ANOVA to find the differences of the data between implant compound and time of implantation. The confidence interval was 95%.

3. Result

3.1 Neutrophil Cell

There was neutrophil cells descriptive on day after implantation, all of the groups show the highest average number of neutrophil cells count. At the first day of implantation, the HA implantation group showed the highest average number of neutrophil cell number compared with CHA implantation group or control group.

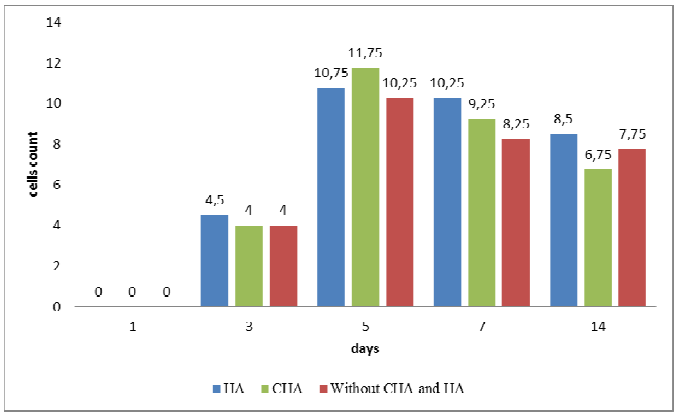
The average number of neutrophil cell count was decrease for the entire group at the third day after implantation and at the 14th day after implantation, the highest average of neutrophil cell number was on control group. Statistically, the average number differences in neutrophil cell wasn't statistically different (p value 0,382).

3.2 Macrophage Cell

The macrophage cell wasn't found at the first day implantation. At the third day after implantation, macrophage cell has begins to show for all groups and the most of macrophage cell was at HA implantation group compared with

average number of macrophage cell count was at the fifth day after implantation for the entire groups and decrease until the 14th days after implantation.

Based on statistical analysis, there was differences in average number of macrophage cell number in CHA implantation group and HA implantation group at the third day after



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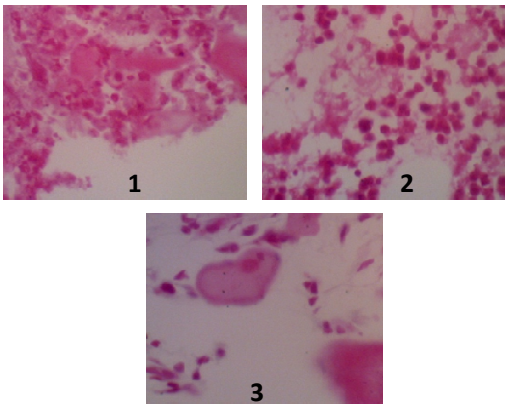

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Figure 2. A) Average number of macrophage cell number of rabbit mandible based on implantation time, B) Histological image of macrophage cell on implantation of CHA (1), HA (2), and without CHA and HA (3).

implantation (p value 0,018), fifth day after implantation (p value 0,000), seventh day after implantation (p value 0,000) and fourteenth day after implantation (p value 0,000). The average number of macrophage cell count of CHA implantation group at the first day was statistically different with control group at the fifth day after implantation (p value 0,000).

The average number of macrophage cell count of CHA implantation group in every implantation periods was lower than HA implantation group except at the fifth day after implantation. Moreover there was no statistically differences between all group in average of macrophage cell count (p value 0,340).

3.3 Edema Volume

At the first day of implantation, edema volume show the highest volume for all group, furthermore, HA implantation groups shows the biggest edema volume (4.19mm^3) compared with other groups. Moreover, the edema volume was decrease at the following days of investigation.

There were statistically differences in edema volume between CHA implantation group at the first day of implantation compared with HA implantation at the third day of implantation (p value 0,000), at the fifth days of implantation (p value 0,000) at the seventh days of implantation (p value 0,000) and the fourteenth days of implantation (p value 0,000). The edema volume in CHA implantation group at the first day after implantation also showed the differences with control group at the third days after implantation (p value 0,000), fifth days after implantation (p value 0,000), seventh days after implantation (p value 0,000) and fourteenth days after implantation (p value 0,000).

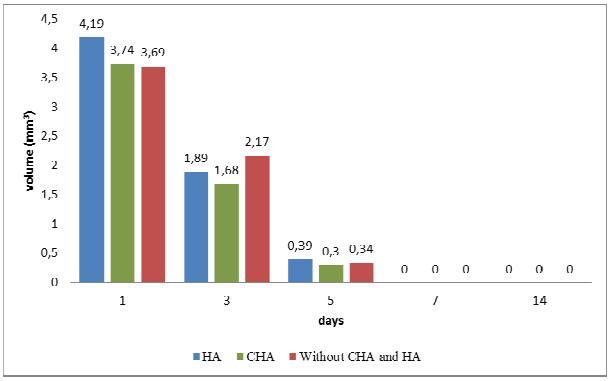

Figure 5. Average of edema volume of rabbit mandible based on implantation time

Statistically, edema volume of CHA implantation group was lower than HA implantation group. Moreover, the statistic results of edema volume's average for all groups was not statistically different (p value 0,199).

4. Discussion

The results of this research showed that neutrophil cell count was highest at the first day after implantation for all groups. This phenomena happened because the inflammation phase was happened in 24 to 48 hours after wound created [15]. This phase was marked by infiltration of

inflammation cells such as polymorfonuclear leukocyte (PMN) cell. The increase of neutrophil cell number was caused by implant material will induce inflammation response. In the HA implantation groups, after HA was implanted into the body, it will induce the increase of several mediator such as interleukin 1α, chemotaxis factor such as interleukin-8 and also matrixs metallo protein (MMP) which will induce the neutrophil activity[16].

Inflammation mediators induced arteriol, capillary, and vena dilatation which will induce leukocyte migration several inflammation area [17]. Phagocytic activity of neutrophil through several mechanisms such as antigen-surface receptor interaction, pseudopodia formation and phagosome, and destruction phase by oxygenase enzyme, lisozyme enzyme, and kationic protein [18, 19, 20].

Neutrophil cell count of CHA implantation groups was less than HA implantation groups. This condition was caused by the existence of carbonate ion in the CHA material. Carbonate increased the bioresorpsabilities of CHA so CHA material had better adaptive ability in the body [21].

At the third days after implantation, neutrophil cell count was decrease for all groups. Phagocytosys activity of neutrophil toward HA was caused by the several cytokine and chemokine such as IL-1α dan IL-8 [16]. Interleukine 1α was a strong activator in many cells including neutrophil cell. Interleukine 1α was part of interleukin 1 which has portion in acute inflammation. Interleukine 1α influenced neutrophil cell activity by induce degranulation and or extracellular inflammation mediator release [22]. Interleukin-8 had chemotactic effect in neutrophil. Interleukin-8 induced leukocyte circulation influx by create concentration gradient between inflamed tissue and blood vessel. Several research showed that HA induced TNFα production [23] or increased oxidative material production by neutrophil [24]. The decreased of neutrophil cell count was happen in all groups from days three after implantation until days 14^{th} after implantation.

At the first day after implantation, the number of macrophage cell count for all groups was zero (0). This phenomena was caused by monocyte will be active and differentiate into macrophage in 48 hours after wound formation [25]. The function of macrophage was for phagocytic and destroy virus, necrotic tissue or foreign material in the body. Macrophage had higher phagocytosys ability than neutrophil [26].

Macrophage begins to show in the 3^{rd} days after implantation. The average number of macrophage cell count was highest at 5^{th} days after implantation for CHA implantation groups. The function of macrophage was to phagocyte bacterial, virus, necrotic tissue or foreign material in the body [26]. In the CHA production process, the temperature will influence the crystallization pattern of CHA, moreover, lower crystallization will make the absorption process into the body will be faster [27].

HA material induced higher inflammation response, it showed by the highest edema volume (4,19 mm^3) compared with other groups. One of the cardinal sign of inflammation response was edema formation. Edema was caused by changes of vascular which is marked by increase of blood flow in the trauma area and the increase of blood vessel permeability [28].

Edema volume was decrease from the third days after implantation and it was not seen any more in the seventh and fourteenth days after implantation. Edema was one of acute inflammation sign which will appear from right after trauma happen until at the first days of trauma. After the first day of trauma, edema will be smaller and in the third days after implantation, the edema will be disappears.

Two ways ANOVA test results and Tukey HSD post ANOVA showed that the average number of neutrophil, macrophage and edema volume was not different for all groups. CHA and HA material was equal based on their main composition. Main composition of both materials was calcium and phosphate [14] which is the main component of human bone formation, moreover it makes CHA and HA material gives an equal inflammation response from the body. The differences of CHA and HA material was on the carbonate addition in CHA material and CHA production process was different also. Carbonate addition will create a different nature of CHA such as increase it solubility and osteoconductivity [6].

The number of neutrophil, macrophage and edema volume after CHA implantation was not show the differences if compared with HA material and control groups. The number of neutrophil, macrophage and edema volume was influenced by the duration of test. Based on those description, inflammation response of CHA implantation was as same as HA implantation or control groups.

5. Conclusion

There was no difference inflammation response between cha and ha implantation based on neutrophil level, macrophage, and edema volume on cha implantation response assay using local rabbits.

Conflict of Interest

The authors report no conflicts of interest

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