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Effects of platelet-rich plasma and carbonated hydroxyapatite combination on cranial defect Bone Regeneration: An animal study

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ABSTRACT

Background: Recently, platelet-rich plasma (PRP) has become popular in the tissue engineering field. PRP has a high concentration of platelets that is three to five times above that of normal plasma and contains several growth factors such as platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor (PDAF), platelet-derived endothelial growth factor (PDEGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). Standard reconstruction of cranial bone defects involves the use of auto- or allogenic biomaterial, such as carbonated hydroxyapatite (CHA) as a scaffold for the osteogenesis process. The purpose of this study is to investigate whether any additional compound may improve the bone healing process.
Methods: This study involved animal experiments on white male rats (*Rattus norvegicus*). Three millimetre diameter were created in rat cranium. Samples were divided into three groups: first group, the cranial defect was grafted with CHA combined with PRP; second group, with CHA alone; and third group, the defect was left as secondary healing wound (control group). The wound healing process was observed for the presence of inflammatory cells and the occurrence of woven bone and lamellar bone. The results among the groups were compared and analysed by the Mann Whitney test using SPSS Statistics Program Package Version 22.0.
Results: The experimental group of 2 weeks showed no difference between inflammatory response ($p = 0.119$), woven bone ($p = 0094$) and lamellar bone ($p = 0130$). At 4 weeks, a combination of PRP and CHA showed a superior growth of lamellar bone compared to CHA ($p = 0008$).
Conclusion: A combination of PRP and CHA as a bone regeneration scaffold showed a histologically increased bone formation.

1. Introduction

Platelet-rich plasma (PRP) application has emerged as a new approach to tissue regeneration and tissue engineering paradigm as a natural source of growth factors that may accelerate bone regeneration [1]. PRP acts as a biomaterial accelerator of bone formation and contains proteins and many growth factors (GF) with osteoinductive characteristics [2]. PRP has a high concentration of plasma platelets which is five times higher than that of the blood (normally 150,000–350,000 cells/μL) and contains at least seven growth factors such as platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor (PDAF), platelet-derived endothelial growth factor (PDEGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) [3]. These growth factors are present within granules inside the concentrated platelets of PRP [4]. Growth factors are proteins that enable different cellular processes involved in tissue healing, such as infiltration, growth, differentiation, migration, cell metabolism and apoptosis. The modification of growth factors may reinforce tissue restoration by upregulating the aforementioned processes [5]. The application of PRP to autogenous bone grafts increases bone mineral density and accelerates bone regeneration and soft tissue

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Table 1
Inflammatory response among the three experimental groups.

	2 Weeks				4 Weeks			
	Minimum	Maximum	Median	Mean	Minimum	Maximum	Median	Mean
PRP + CHA	1	4	4	3	2	3	2.5	3
CHA	4	4	4	4	3	4	4	3.8
Control	4	4	4	4	3	4	3	3.2

PRP: platelet-rich plasma, CHA: carbonate-substituted hydroxyapatite.

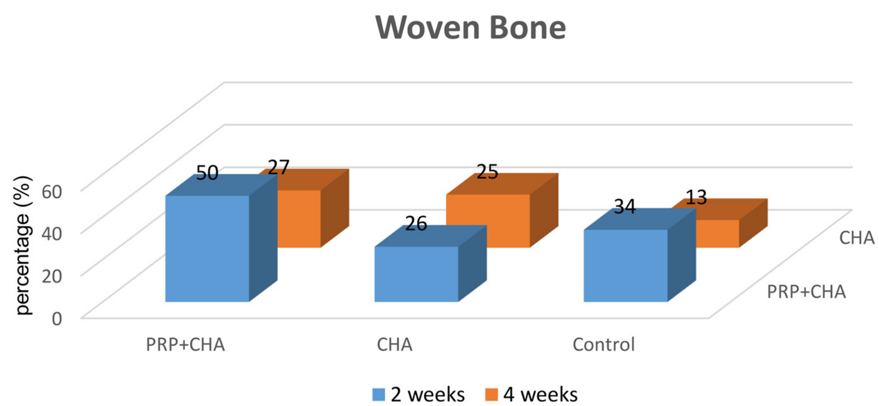

Fig. 1. Percentage of woven bone occupying the cranial defect. PRP: platelet-rich plasma, CHA: carbonated hydroxyapatite.

healing. PRP has been successfully used for mandibular reconstructions and dental implant procedures [6].

Bone healing and regeneration is one of the most important processes in craniofacial surgery. Carbonate-substituted hydroxyapatite (CHA) is a major inorganic component of natural bone, and owing to its bioactive, biodegradable and osteoconductive properties, CHA has been used extensively in biomedical implant and bone regeneration applications. CHA is also known to be biocompatible, non-toxic, non-inflammatory, and non-immunogenic, and is capable of forming a direct chemical bond with surrounding hard tissues. CHA is synthesized from calcium nitrate tetrahydrate, diammonium hydrogen phosphate and sodium hydrogen carbonate. CHA is also more osteoconductive and more resorbable than hydroxyapatite (HA) [7]. Carbonated HA contains 3–5% carbonate ions by substitution in the hydroxyapatite lattice structure and is the major mineral constituent of bone [8]. Several studies such as Ellies et al. [9] and Patel et al. [10] have reported improvement of bone formation with CHA implants compared with HA controls.

The present study aimed to evaluate the effects of PRP and CHA on bone regeneration in experimental rat cranial defects.

2. Methods

The study has been approved by scientific study ethical committee of Universitas Sam Ratulangi. The present study was an animal experimental study using 36 male rats (*Rattus norvegicus*) aged 20–22 weeks old and with body weight of 350–400 g. The animals were kept individually in quarantined rooms under 12-h light/dark cycle with controlled temperature and humidity, and minimal noise, and they had full access to standard dry food and water ad libitum. Each experimental group consisted of six rats. The rats were anaesthetized with an intramuscular injection of ketamine 10% (30 mg/kg body weight), and their head was then shaved over the cranium and draped in a sterile manner. Following a nasofrontal-external protuberance occipital approach, the periosteum was dissected and identical full-thickness bony defects were created with a 3-mm round bur. The defects were filled with the combination of PRP and CHA (group 1), CHA alone (group 2), and kept unfilled to serve as a control (group 3). The wounds were closed with a non-absorbable 5/0 suture.

2.1. PRP preparation

A total of 3 mL autologous blood was drawn from each rat and combined with the anticoagulant citrate dextrose phosphate to prevent coagulation. The blood sample was then centrifuged at 160*g* for 20 min to separate the plasma containing the platelets from the red blood cells. The plasma was extracted from the top and centrifuged for an additional 15 min at 400*g* to separate the platelets. The platelet-poor plasma was separated from the PRP along with the buffy coat. The rats were euthanised with an overdose of diethyl ether at 2 and 4 weeks. The entire cranium was removed with a reciprocating saw. Specimens were treated with 20% formic acid decalcifying solution for three days, dehydrated with alcohol and then embedded in paraffin. The specimens were prepared routinely with haematoxylin and eosin staining. Histologic evaluation was performed at 10 times magnification.

2.2. Outcomes

After two weeks of treatment, the wound healing process was examined for the inflammatory response as follows: < 5 leucocyte/field of view (grade 1); 5–10 leucocyte/field of view (grade 2); 10–50 leucocyte/field of view (grade 3); > 50 leucocyte/field of view (grade 4) at 2nd and 4th weeks. The other outcome measurement was bone formation (woven bone and lamellar bone; percentage occupying the defect), and the value were compared between the groups at 2nd and 4th weeks.

2.3. Statistical analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) version 22.0. One-way analysis of variance (ANOVA) and Kruskal Wallis test were performed for statistical analysis. A p value of less than 005 was considered statistically significant.