INFLAMMATION RESPONSE OF MECHANICALLY EXPOSED PULP AFTER DIRECT PULP CAPPING WITH CALCIUM HYDROXIDE CEMENT AND PLATELET RICH PLASMA

(RESPONS PERADANGAN PADA PULPA TERBUKA SECARA MEKANIK SETELAH PULP CAPPING DIRECT DENGAN SEMEN KALSIUM HIDROKSID DAN PLATELET RICH PLASMA)

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Abstract

During cavity preparation and the removal of caries dentine, it is possible to accidentally expose the dental pulp. Direct pulp capping may be indicated for maintaining pulp health and function. The direct pulp capping material that used in this study was platelet rich plasma (PRP). Platelet rich plasma is on autologous growth factor containing of platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), insulin growth factor (IGF) and epidermal growth factor (EGF). The aim of this study was to evaluate inflammation response of pulp tissue following direct pulp capping with PRP. Thirty sound teeth from Sprague Dawley were used and divided into two groups: group 1 as a control, teeth were capped with calcium hydroxide/$Ca(OH)_2$ (n=15) and group 2 with PRP (n=15). After the 1st, 7th and 21st days, respectively, 5 teeth of each group were processed for light microscopic examination. Inflammation responses were assessed by haematoxylin eosin. The result showed a similar inflammation response of $Ca(OH)_2$ (group 1) on the 1st and 7th days 40% necrose and 40% severe inflammation and the 21st day 60% samples were severe inflammation. Inflammation response of PRP (group 2) on the 1st day showed 40% necrose and 40% severe inflammation, on the 7th day, 100% severe inflammation and on the 21st day, 60% mild inflammation. After the 21th day observation period, Kruskal Wallis test showed that inflammation response was not significant in both groups ($p>0,05$). In conclusion, PRP and $Ca(OH)_2$ as a direct pulp capping material had no different inflammation response.

Key words: *pulp capping, platelet rich plasma, inflammation response*

INTRODUCTION

During the process of cavity preparation and the removal of carious dentine it is possible to happen the result in iatrogenic exspose pulp.[1] Pulp capping is a dental vital treatment in order to maintain integrity, morphology and function of the pulp. There are two kinds of pulp capping, indirect and direct pulp treatment. Indirect pulp capping treatment is indicated for deep dental carious and there is still a layer of dentine. Clinically and radiologically there are no pulpal degeneration and periradiculer disease. Direct pulp capping treatment is indicated for open pulp due to trauma or restoration procedure.[2]

Calcium hydroxide is a pulp capping material used for a long time and widely in endodontic treatment in the field of dentistry, because of it's ability to change the environment for tissue healing.[3] Some researchers report the successful treatment with calcium hydroxide[3] but Suardita reveals several studies indicating that there is a lack of calcium hydroxide as pulp capping material, including necrotic area forming in the direct contact with the material.[4]

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Some researchers develop PRP as a pulp capping material. And one of them is Kim et al, revealing that the application of PRP in the injury healing process gives better results than the application of the growth factor alone.[5]

PRP is a natural source of autologous growth factors and contains of growth factors such as platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), insulin growth factor (IGF) and epidermal growth factor (EGF).[6] Carlson and Roach stated that the PRP can accelerate healing in maxillofacial and periodontal surgery in cases of maxillofacial reconstruction, implant and bone graft.[7]

The healing process of pulp exposed includes in the processes of inflammation.[8] Inflammation process is a response to injury and pulpal defense mechanisms needed to improve the structure and function of the pulp tissue. Inflammation response in the pulp tissue is marked by infiltration of polymorphonuclear leukocyte cells at the site of exposure.[9]

Based on the background, it can be formulated that the problem is what is the comparison of inflammation response in the mechanically exposed pulp after direct pulp capping with calcium hydroxide ($Ca(OH)_2$) and platelet rich plasma (PRP).

MATERIALS AND METHODS

Before conducting the study, ethical clearance was prepared and it's issued by the Medical Research Ethics Committee of Health, Gadjah Mada University. Subjects were 30 teeth, divided into two groups consisting of 15 teeth as a treated group (PRP) and 15 teeth as a control group ($Ca(OH)_2$). Each group was divided by the observed period on the 1st, 7th and 21st days. The criteria of this study is tooth of *Sprague Dawley*, that are 250-300 grams and 3-4 months.

The preparation of platelet rich plasma (PRP) was made in the Laboratory of Clinical Pathology Faculty of Medicine, University of Gadjah Mada Yogyakarta, by doing gradient centrifugation.[6] Before platelet rich plasma applied as a pulp capping material, it's mixed with 10% calcium chloride in sterile saline and 100 u/ml sterile bovine thrombin in the same volume. This mixture of PRP is sticky gel so it is relatively easy to apply.[1]

Before the preparation, animals were anaesthetized using chloral hydrate 8% at a dose of 350 mg/kg body weight. Molar teeth of the upper right and left experimental animals were prepared on the occlusal surface using a round bur no 10 with a diameter of 1 mm^2 and a depth of 1 to 1.2 mm to approach the pulp then it was performed with the sonde to the pulp perforation open.

On days 1st, 7th and 21st, decapitation was done. Experimental animals were anaesthetized with ether and cervical dislocation done. Tooth samples extracted and fixed with 10% buffered formalin for 24 hours. Then they performed decalcification with formic acid for 1 week. After that they were planted in paraffin and then cut longitudinally with a thickness of 4μm and stained by hematoxylin eosin (HE).

Data were analysed statistically by using Kruskal Wallis test (SPSS 10.0 software, spss, Chicago, IL, USA) to find out whether there were significant differences on the inflammation response in the control and the treatment groups, according to histological grading criteria of Table 1.

Table 1. Inflammation cell response

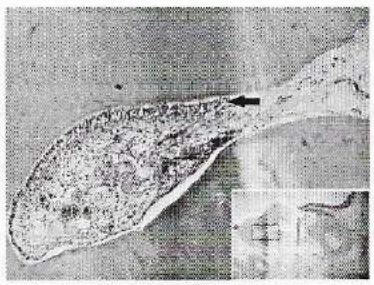
Grade	Characterization
0	No inflammation in the affected pulp injury and has the characteristics of normal pulp tissue
1	Mild inflammation response characterized by polymorphonuclear leukocytes in the affected lesion
2	Moderate inflammation response is characterized by inflammation cells found in the coronal pulp
3	Severe inflammation response characterized by cellular infiltration in the coronal pulp with abscess formation
4	The network suffered/pulp necrosis

RESULTS

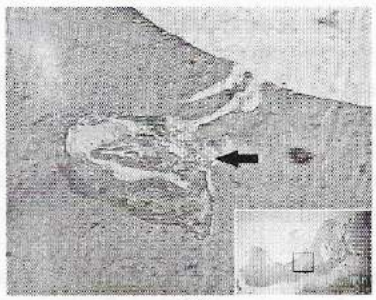
Table 2 showed groups of $Ca(OH)_2$ on the 1st and 7th days had the same inflammation response. 40% of samples had inflammation seen by light microscopic. There were infiltrating polymorphonuclear leukocytes extended to the coronal (Fig. 1A), and 40% of samples had necrosis (Fig. 1B). After pulp capping with $Ca(OH)_2$ on the 21st day 60% of samples had severe inflammation, there was infiltration of PMN leukocytes reaching the pulp with abscesses (Fig. 1C).

Table 2. Score of inflammation responses towards the pulp exposed after direct pulp capping with $Ca(OH)_2$ and PRP in each observation period on the 1st, 7th and 21st days

Score	Ca(OH)2			PRP		
	1st day (%)	7th day (%)	21st day (%)	1st day (%)	7th day (%)	21st day (%)
0	0	0	0	0	0	0
1	0	0	0	0	0	0
2	2 (40)	2 (40)	0 (0)	1 (20)	0 (0)	3 (60)
3	1 (20)	1 (20)	3 (60)	2 (40)	5 (100)	1 (20)
4	2 (40)	2 (40)	2 (40)	2 (40)	0 (0)	1 (20)



(A)



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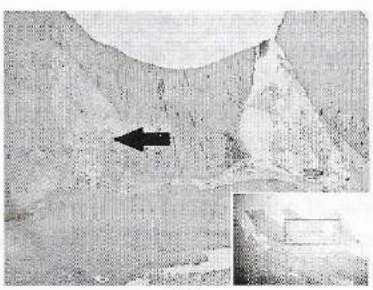
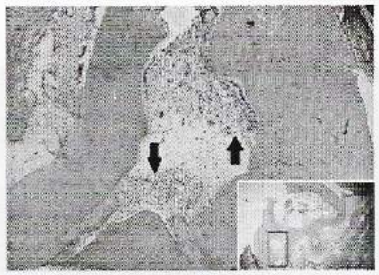


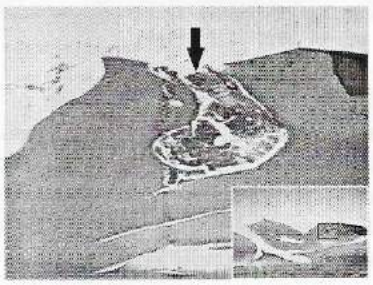
(C)

Figure 1. Light microscopic examination of the inflammation response by HE staining (magnification 200x). (A) application of $Ca(OH)_2$ on the 1st day with category of moderate inflammation, there was infiltration of PMN (arrow). (B) application of $Ca(OH)_2$ on the 1st day with a category of necrosis (arrow). (C) application of $Ca(OH)_2$ on the 21st day with category of severe inflammation (arrow)

The inflammation response of pulp exposed after pulp capping with PRP on the 1st day was 40% severe inflammation that was characterized by PMN infiltration in the coronal pulp with abscesses (Fig. 2A) and 40% of samples had necrosis (Fig. 2B), 20% of samples had severe inflammation response, They were characterized by cellular infiltration in the coronal pulp with abcess formation (Fig.1C).



(A)

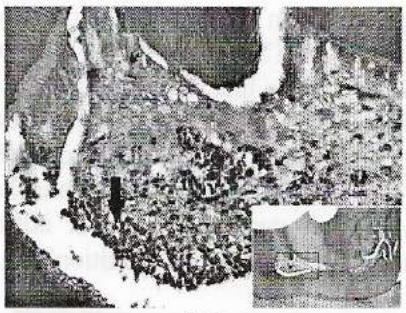


(B)

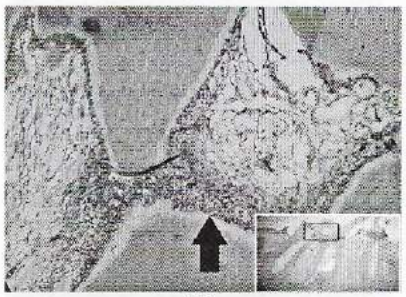
Figure 2. Light microscopic examination of the inflammation response by HE staining (magnification 200x) on the 1st day after direct pulp capping with PRP. (A) On the 1st day application of PRP with severe inflammation category (arrow). (B) The 1st day application of PRP with necrosis category (arrow)

On the 7th day after direct pulp capping with PRP, 100% of the samples were severe inflammation characterized by PMN infiltration reaching the coronal with abscess formation (Fig.3A) and on the 21st day, 60% of samples had moderate inflammation that was characterized by PMN infiltration in the coronal pulp (Fig.3B).

There was no significant difference in the application of calcium hydroxide and PRP as a direct pulp capping material on the 1st, 7th and 21st days on the inflammation response in the pulp exposed ($p>0,05$).



(A)



(B)

Figure 3. Light microscopic examination of the inflammation response by HE staining (magni-fication 200x). (A) PRP application on the 7th day category of severe inflammation (arrow). (B) PRP application on the 21st day category of moderate inflammation (arrow)

DISCUSSION

Table 2 showed there was inflammation response that tended to vary in the application of $Ca(OH)_2$ as a direct pulp capping on the 1st, 7th and 21st days. It is possible because $Ca(OH)_2$ had a high pH (12.5). Calcium ions can increase the environmental effect of alkali and give effect of sterilization so that $Ca(OH)_2$ has the effect of bacterisid. $Ca(OH)_2$ has the ability to release ions Ca^2 and OH^- differently in each preparation so it produces different inflamemation response.[3]

Mechanism of $Ca(OH)^2$ and PRP as a pulp capping is different in responding of inflammation. Calcium hydroxide as a pulp capping material can response injury because of alkaline serves bakterisid[11], while the PRP has a function as a mediator of biological processes that control the inflammation during the process of exposed pulp tissue repairment.[6]

In conclusion, calcium hydroxide ($Ca(OH)_2$) and platelet rich plasma (PRP) as a direct pulp capping material has no different inflammation response in the pulp exposed. It needs further research on the specifications of composition and concentration of PRP as an ingredient of pulp capping.

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