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Research Article

The Effect of Allogenic Freeze Dried Platelet- Rich Plasma in Immunological Responses of Rabbits

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Abstract: This study aims to analyze the effects of allogenic freeze-dried platelet-rich plasma (PRP) in immunological responses of rabbits. This study employed a design of conducting one pre-post test group to determine the effect of freeze drying on levels of TGF-β1 PRP and the post test design was only for control group conducted to determine the effect of allogenic freeze-dried PRP. Levels of TGF-β1 before and after freeze drying from nine samples of PRP were examined which were obtained from blood centrifugation of three rabbits. These nine samples were used as allogenic donor injected intramuscularly in nine rabbits for the treatment groups. The control group used nine rabbits injected intramuscularly using autologous PRP. Inflammatory response and increasing levels of IgM were observed from both groups resulted in data which were then tested statistically using independent T-test. Measurement of TGF-β1 levels before and after freeze drying were tested statistically using T- test dependent. The results showed that freeze drying process did not affect levels of TGF-β1. Allogenic freeze-dried PRP did not cause an inflammatory response in addition to not increasing levels of IgM.
Keywords: autologous, allogenic, freeze dried platelet rich plasma, transforming growth factor -β1, IgM.

INTRODUCTION

The use of platelet-rich plasma (PRP) in tissue regeneration has developed as the more number of research and application in the clinical. Platelets are fragments formed cytoplasm of megakaryocytes in the bone marrow. Platelets are the smallest parts of the blood cells, round or oval and with no core, but contain a number of organelles. The structure consists of mitochondria, microtubules and 50-80 grains of granules (α, δ, λ). A granular α has more than 30 bioactive proteins, chemokines and various growth factors such as Transforming Growth Factor β (TGF-β), Platelet Derived Growth Factor (PDGF), vascular endothelial growth factor(VEGF), insulin-like growth Factor (IGF), Fibroblast growth Factor (FGF) [3]. These growth factors can be used for tissue repair therapies in various branches of medicine such as oral surgery, plastic surgery, craniofacial surgery, cardiac surgery, orthopedics, neurology, sports medicine, and dermatology [2].

In the case of tissue repair, growth factor such as TGF-β stimulates fibroblasts and increases extra cellular matrix formation (ECM). Transforming Growth Factor – β also increases collagens for wound healing process. Other growth factor such as PDGF also functions in the repair of bone tissue by stimulating the production of collagen type1, which induces the synthesis of bone [5]. In the process of tissue repair tendon IGF can stimulate the proliferation and differentiation of myeloblast [4].

In general, platelets are used for clinical applications derived from the patient's own (autologous). The use of autologous products can eliminate immunologic reactions and disease transmission [1]. However, autologous therapy cannot be performed in patients with a deficiency or abnormality of platelet function. This leads to the use of autologous in large variability, because the platelet concentration varies between individuals. Thus, it is difficult to evaluate the results scientifically [6]. Some patients also do not have the courage to do blood sampling in large numbers. Therefore, the use of allogenic PRP (derived from other individuals within a species) is needed as an alternative to growth factor therapy.

This study aims to analyze the effects of allogenic freeze-dried platelet-rich plasma in immunological responses of rabbits. The immunological response were observed from inflammatory response and increasing level of IgM

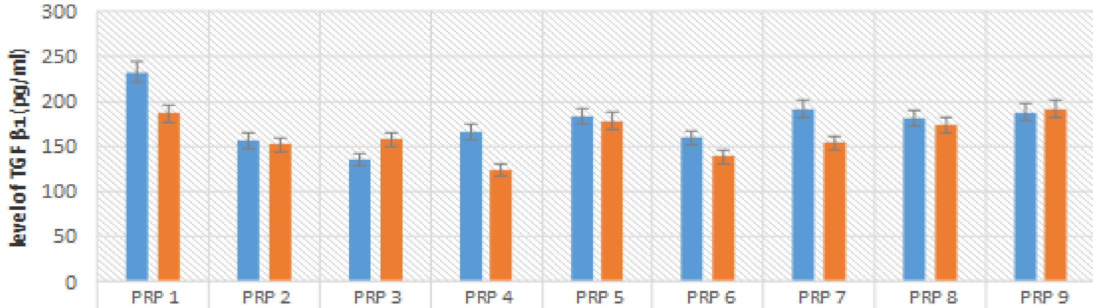
MATERIALS AND METHODS

This study was conducted under permission of Animal Care and Use Committee (ACUC) Airlangga University. The first step was using nine samples of PRP with measured TGF – β1 before and after freeze drying process. The second step was with two groups, control and treatment groups. The control group used nine rabbits injected intramuscularly using autologous PRP whereas the nine rabbits in the treatment group were injected intramuscularly using allogenic freeze-dried PRP. Inflammatory response and increasing levels of IgM were observed in both groups. Measurement of TGF-β1 levels before and after freeze drying were tested statistically using dependent T- test, while that of the independent was employed for statistically testing data of inflammatory response and increasing levels of IgM.

RESULTS

Levels of TGF - β1 Before and After Freeze Drying Process

The results showed that the highest level of TGF-β1 at PRP before freeze drying process was 232 pg / ml and that of the lowest was 136 pg / ml, with the mean of 178 pg / ml and SD ±27 pg / ml. Meanwhile, the highest level of TGF-β1 in PRP after freeze drying process was 192 pg / ml and the lowest was 124 pg / ml, with the mean of 162 pg / ml and SD 23 pg / ml. From statistical decision of 0.081 > 0.05, it can be concluded that the level of TGF-β1 in PRP before and after freeze drying process was not significantly difference.



	PRP 1	PRP 2	PRP 3	PRP 4	PRP 5	PRP 6	PRP 7	PRP 8	PRP 9
before	232	157	136	167	184	160	192	182	188
after	187	153	158	124	179	139	155	174	192

Fig-1: Levels of TGF - β1 Before and After Freeze Drying Process

Evaluation of inflammatory reactions

Table 1. Data rabbit body temperature control and treatment groups after intramuscular injection

Autologous PRP	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6	Control 7	Control 8	Control 9
Body temperature (^{0}C)	38.5	38.3	39.1	38.8	39.1	39.2	38.9	38.3	38.5
Allogenic freeze dried PRP	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Body temperature (^{0}C)	39.3	38.5	39.2	38.9	38.7	39.2	38.5	38.6	39.1

Referring to data on rabbit body temperature in control and treatment groups after intramuscular injection, statistical results were 0,379 > 0.05 meaning that there was no difference in body temperature in the control group (autologous) and treatment group (freeze-dried allogenic PRP). Thus, the use of allogenic freeze-dried PRP did not cause inflammatory reactions / inflammation.

Evaluation the levels of IgM

Table 2: Data IgM levels of control and treatment groups after intramuscular injection

Autologous PRP	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6	Control 7	Control 8	Control 9
Level IgM (ng/ml)	20	39	79	132	35	36	23	46	53
Allogenic freeze dried PRP	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Level IgM (ng/ml)	22	64	99	59	87	73	96	102	112

Statistical results for data on IgM levels of control and treatment groups after intramuscular injection were 0.080 > 0.05. This implied that there was no difference in the levels of IgM formed in the control group (autologous) and treatment group (allogenic freeze-dried PRP). It can be concluded that the use of allogenic freeze- dried PRP did not induce humoral immune response in the form of primary antibodies (IgM).

DISCUSION

Allogenic PRP used in this study first performed the process of freeze drying. The purpose of the freeze drying process is to preserve the content of growth factors contained in PRP. Several studies have been conducted to determine the advantages of the use of freeze drying in the process of preserving a material or substance which is susceptible to heat like growth factor of TGF-β1. Another study showed that freeze drying process did not affect the concentration of TGF-β2 contained in the freeze- dried amniotic membrane. This is evidenced from the results of this research showing no significant difference between the concentrations of total TGF-β2 in the form of fresh amniotic membrane and freeze-dried [7].

Although platelet is not a cell with a nucleus, the membrane surface contains a number of family immune molecules such as MCH Class I, Fc receptors and complement binding site. Platelet membrane also contains antigens of human platelet antigen (HPA) and antigen of polysaccharide ABO blood type to another, where it was very important in blood transfusion system [6]. Based on the theory of the use of allogenic platelets, it can cause an immune response.

Zhang have proposed that allogenic PRP used in gel PRP did not increase immune response. There were no increase in the level of CD4 and CD8, so it may cause the gel covered surface of platelet contains HPA [8]. Allogenic PRP used in this study first conducted the process of freeze drying. The existence of the freezing process – 83^0C for 24 hours in freeze drying process raised an assumption that it can damage the structure of immunoglobulin protein molecules and antigen found on the surface of the platelet membrane. So, the non reactive antigens did not cause an inflammatory response and did not increase levels of IgM, either.

CONCLUTION

Regarding this, the product allogenic freeze dried PRP is safe for clinical applications.

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