

Effect of Platelet Rich Plasma (PRP) on Proliferation of Endothelial Progenitor Cell (EPC) of Stable Coronary Artery Disease Patient

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Abstract

Background: Endothelial Progenitor Cell (EPC) is the progenitor of endothelial cell which has important role in regulation of vascular wall integrity and homeostasis, to protect vessels from inflammation and thrombosis, that leads into pathogenesis of coronary artery disease. Growth factors proven has important role to stimulate transduction signal in the process of proliferation of EPC. Platelet Rich Plasma (PRP) contains variety of growth factors, wellknown role in homeostasis and wound healing process. Therefore, this study was conducted to analyze the effect of PRP on proliferation of EPC of Stable Coronary Artery Disease (SCAD) patient. To analyze the effect of Platelet Rich Plasma (PRP) on the proliferation of Endothelial Progenitor Cell (EPC) from peripheral blood of patient with SCAD

Methods: This is an in vitro, true experimental, post-test only control group design. The mononuclear cells were isolated from peripheral blood of SCAD patient and cultured in M-199 medium. EPC divided into 3 groups, which received Platelet Rich Plasma (PRP), Platelet Poor Plasma (PPP), and control. After 14 days of incubation, immunocytochemical examination was performed, EPC which marked with CD34, FITC labeled, was counted using immunofluorescence microscope. Data analysis using ANOVA test.

Results: Cell counting showed significant increase of EPC proliferation in PRP group compared to PPP group (1.052 ± 0.16 vs 0.762 ± 0.19 , $p = 0.003$), and control group as well (1.052 ± 0.16 vs 0.068 ± 0.05 , $p = <0.001$). EPC proliferation in PPP group also increase significantly compared to control group (0.762 ± 0.19 vs 0.068 ± 0.05 , $p = <0.001$).

Conclusion : Platelet Rich Plasma (PRP) increase EPC proliferation significantly from peripheral blood of SCAD patient.

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Keywords : EPC proliferation, PRP, SCAD

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Introduction

Coronary artery disease (CAD) has high prevalence for morbidity and mortality rate worldwide. Current therapies for this cardiovascular diseases (CVD) include lifestyle management, pharmacological control for risk factors and revascularization, have improved the survival but not completely control the disease,

therefore new approaches like cell-based therapy are needed¹. Endothelial cell dysfunction is condition that lead to pathologic atherosclerotic in CAD patients. An increasing evidence suggest that cardiovascular risk factors affect the number and properties of EPC, the inverse correlation is found between both². In 1997, Asahara and his colleagues first isolated mononuclear cell (MNC) that express CD34 and VEGF-2 from human peripheral blood, named Endothelial Progenitor Cell (EPC)³. This cells play an important role in the recovery and repair of injured endothelium by counteracting the detrimental CVD risks factor induced damages. Growth factors has proven role in stimulating transduction signal for proliferation of EPC and promote neovascularization⁴. Platelet Rich Plasma (PRP) which has minimum 5 times higher of platelets compared to baseline values for whole blood, has been used clinically in humans since 1970s for its healing properties attributed of autologous growth factors (GF) that may enhance the healing process on a cellular level⁵. Platelet also contains the angiogenic GF that believed promote the neovascularization process. So in this research, we would like to know the effect of PRP on proliferation of EPC in peripheral blood of stable coronary artery disease (SCAD).

Methods

Blood samples were taken from patients with SCAD which are man, had narrowing of $\geq 50\%$ in the left main coronary artery and $\geq 70\%$ in one or several of the major coronary arteries from angiography. Patients with history of acute myocardial infarction, acute limb ischemia, or after revascularization therapy include stent placement or coronary artery bypass grafting (CABG) were excluded.

Written informed consent was obtained from patients before peripheral blood drawing. The protocol was approved by local ethics committee (0421/KEPK/VII/2018).

Preparation of PRP and Platelet Poor Plasma (PPP)

Peripheral blood (20 mL) was collected from SCAD patient using anticoagulant, citrate-phosphatedextrose solution. Collected blood was centrifuged at 2400 rpm

for 10 minutes at 200C and the supernatant (plasma) including the buffy coat and 0.5 mL below buffy coat was decanted to the other tube and red blood cells (RBCs) and white blood cells (WBCs) were discarded. Secondary centrifugation was performed at 3600 rpm for 10 minutes at 20⁰C. The clear supernatant (plasma) was decanted off until 0.5 mL was left and the middle portion of supernatant (plasma) was taken as PPP. Finally, remaining supernatant including buffy coat and 0.5 mL below buffy coat was taken as PRP⁶.

Isolation and culture of EPCs

Peripheral blood from SCAD patient (40 ml) was diluted with phosphate buffered saline (PBS) containing 2% of fetal bovine serum (FBS). It was then centrifuged on Ficoll-Histopaque density gradients and interface mononuclear cells (MNCs) were collected. After two washes in PBS containing 2% of FBS, pellet were taken and cells counted using hemocytometry. Each 5×10^6 cells/ml mononuclear diluted with M-199 stemcell expansion medium were plated on 24-fibronectin coated well plates cells, divided in to 3 groups with different treat (PRP 20%, PPP 20% ,and Control). Every group also given antibiotics (Penicillin and Streptomycin). Cells then incubated for 14 days at 37 °C and 5% of CO₂.

Immunofluorescence assay

Adherent cells were taken and washed with PBS and fixed with 3% formaldehyde for 15 minutes. Cells were stained with a FITC anti-CD34 antibody. Expression of the cells was documented with fluorescence microscope.

Statistical analysis

Data in normal distribution were reported as mean \pm SD. To analyse differences for normally distributed data, one-way ANOVA was used. Differences were considered significant if p-values were <0.05. All statistical analyses were performed with SPSS for Windows (IBM Corp., Armonk, NY).

Results

PRP increase the proliferation of EPC

In this study, Endothelial Progenitor Cells (EPC) which positive stained by CD34+ and checked by immunofluorescence microscope (Figure 1), compared among groups treated with PRP, PPP, and control. The result showed significant increase in group treated with PRP compared to PPP in (1.052 ± 0.16 vs 0.762 ± 0.19 , $p = 0.003$) and significant increase compared to control group as well (1.052 ± 0.16 vs 0.068 ± 0.05 , $p = <0.001$). EPC proliferation in PPP group also significantly increase compared to control group (0.762 ± 0.19 vs 0.068 ± 0.05 , $p = <0.001$).

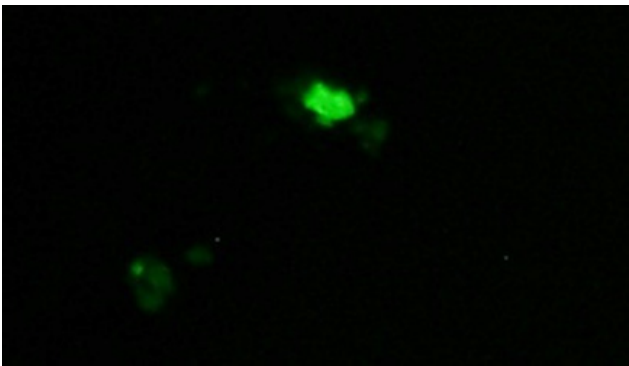


Figure 1. Immunofluorescence expression of CD34

Discussion

The result of the study showed that PRP increase the proliferation of EPC significantly compared to PPP and control group. It has been recognized that plateletes in PRP secreted various growth factors during the degranulation. Different growth factors in PRP, including vascular endothelial growth factors (VEGF), platelet-derived growth factors (PDGF), epithelial growth factors (EGF), and transforming growth factor beta 1 (TGF- β 1), have different role in angiogenesis and restoration of blood flow following ischemia. VEGF is the principal stimulatory factor of angiogenesis after ischemia⁷. VEGF causes endothelial cell proliferation and migration, which result in capillary sprouting or angiogenesis. The stimulation of PRP on angiogenesis is regulated by activation of PI3 kinase and extracellular signal-regulated kinase (ERK)⁸. However,

neovascularization is a complex event, and interplay between cells and angiogenic factors, not VEGF only, needed to form a stable capillary vessel⁹. The late issue is how to optimize the potency of growth factors contained in PRP. Bir et al, shown the efficacy of sustained release of PRP in therapeutic neovascularization and this potentiation is triggered by stimulation of angiogenesis, arteriogenesis, and vasculogenesis in mouse hind limb ischemia¹⁰. We will need more studies to improve the potency of PRP in proliferation and differentiation.

Conclusion

The results of this study define that PRP increases EPC proliferation from peripheral blood of SCAD patient compared to PPP and control group.

Ethical Clearance

This study was approved by the ethic committee of Airlangga University.

Conflict Of Interest

None

Publication Agreement

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Ethical Clearance

0421/KEPK/VII/2018 from Research Ethics Committee, Dr. Soetomo Hospital, Surabaya.

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List of Abbreviations

CABG : Coronary artery bypass graft
CAD : Coronary artery disease
CD : Cluster of differentiation
CVD : Cardiovascular disease
EGF : Epitelial growth factors
EPC : Endothelial progenitor cell
ERK : extracellular signal-regulated kinase
FBS : Fetal bovine serum
GF : Growt factor
MNC : Mononuclear cell
PBS : Phosphate buffer saline
PDGF : Platelet derived growth factor
PI3K : Phosphatidylinositol-3 Kinase
PPP : Platelet poor plasma
PRP : Platelet rich plasma
RBC : Red blood cell
SCAD : Stable coronary artery disease
TGF β -1 : Transforming growth factor beta 1
WBC : White blood cell
VEGF : Vascular endothelial growth factor

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