

ABSTRACT

The Effect of Additional Platelet Rich Fibrin (PRF) on Adipose derived Mesenchymal Stem Cell (AMSCs) Differentiation into Cardiomyocytes In Vitro

Arisya Agita., I Gde Rurus Suryawan, Andrianto

Background: Coronary artery disease among other cardiovascular disease strongly influenced the quality of life. Whereas human adult cardiomyocytes has limited capacity for regeneration. The irreversible loss of cardiomyocytes can lead to progressive ventricular remodeling of nonischaemic myocardium. Clinically there is no treatment to regenerate the infarcted myocardium. On this basis, cell therapy is an ideal treatment to regenerate the damaged cardiac area. Obtaining adipose-derived stem cells (AMSCs) increases yields and reduces the pain in a simple procedure compared to BMSCs. And platelet rich fibrin (PRF) is the newest revolution of platelet therapy which appears to have the ability to induce cardiomyocytes differentiation.

Objective: To analyze the effect of additional PRF on the AMSCs differentiation into cardiomyocyte compared with the group without additional PRF.

Methods: This study is a true experimental randomized post-test design study. AMSCs were isolated from adipose tissues and cultured until 4 passages. The characteristics of AMSCs were measured by the expression of CD 34-, 45-, and CD 105+ using flowcytometry. The samples were divided into 3 groups, i.e. negative control (α -MEM), positive control (differentiation medium) and treatment group (PRF). The assessment of GATA-4 marker expression was conducted using flowcytometry on the fifth day and cTnT was conducted using immunocytochemistry on the tenth day to determine the differentiation to cardiomyocyte. Data analysis was conducted using T-test and One-Way ANOVA on normally distributed data determined through Shapiro Wilk test.

Results: Flowcytometry on GATA-4 expression revealed significant difference on PRF group compared with negative and positive controls (68.20 ± 6.82 vs 58.15 ± 1.23 $p < 0.05$; 68.20 ± 6.82 vs 52.96 ± 2.02 $p < 0.05$). This was supported by the results of immunocytochemistry on troponin expression which revealed significant difference between PRF group compared with negative and positive controls (50.66 ± 7.2 vs 10.73 ± 2.39 $p < 0.05$; 50.66 ± 7.2 vs 26.00 ± 0.4 $p < 0.05$). This was in line with the hypothesis which stated that there was an effect of additional PRF on AMSCs differentiation into cardiomyocytes.

Conclusion: Additional PRF on AMSCs differentiation significantly improve the differentiation into cardiomyocytes measured by GATA-4 and cTnT expressions.

Keywords: Adipocyte-derived mesenchymal stem cells, platelet rich fibrin, growth factor, stem cell therapy

DAFTAR ISI

SAMPUL DEPAN.....	i
LEMBAR PRASYARAT GELAR.....	ii
LEMBAR PENGESAHAN	iii
PERNYATAAN PENELITIAN KARYA AKHIR	iv
PERNYATAAN PERSETUJUAN PUBLIKSI TUGAS AKHIR UNTUK KEPENTINGAN AKADEMIS	v
KATA PENGANTAR	vi
ABSTRAK	xi
ABSTRACT	xiii
DAFTAR ISI.....	xiii
DAFTAR GAMBAR	xvi
DAFTAR TABEL.....	xvii
DAFTAR SINGKATAN	xviii
BAB I PENDAHULUAN	1
1.1 Latar Belakang Masalah	1
1.2 Rumusan Masalah	3
1.3 Tujuan Penelitian.....	4
1.3.1 Tujuan Umum	4
1.3.2 Tujuan Khusus	4
1.4 Manfaat Penelitian.....	4
1.4.1 Manfaat Akademik.....	4
1.4.2 Manfaat Klinis.....	5
BAB II TINJAUAN KEPUSTAKAAN.....	6
2.1 <i>Stem cell</i>	6
2.2 <i>Adipocyte-derived Mesenchymal Stem cells (AMSCs)</i>	7
2.2.1 Karakteristik dan Imunofenotipe AMSCs.....	9
2.2.2 Isolasi dan Kultur <i>Adipocyte Derived Mesenchymal Stem cells</i> <i>(AMSCs)</i>	9
2.2.3 Aplikasi <i>Adipocyte Derived Mesenchymal Stem cells (AMSCs)</i> dalam Regenerasi Otot Jantung.....	11
2.2.4 Diferensiasi AMSCs menjadi Kardiomyosit	11
2.3 Diferensiasi Kardiomyosit	12
2.4 Platelet Rich Fibrin.....	14

2.4.1	Preparasi <i>Platelet Rich Fibrin</i> (PRF)	15
2.4.2	Pengaruh Biologis Platelet Rich Fibrin (PRF)	17
2.4.3	Pengaruh PRF pada Bidang Kardiovaskular	19
BAB III KERANGKA KONSEPTUAL		22
3.1	Kerangka Konseptual	22
3.2	Hipotesis Penelitian	24
BAB IV MATERI DAN METODE PENELITIAN		25
4.1	Rancangan Penelitian	25
4.2	Tempat dan Waktu Penelitian	26
4.3	Materi Penelitian	27
4.4	Besar Sampel	27
4.5	Teknik Randomisasi	27
4.6	Variabel Penelitian	28
4.6.1	Variabel Bebas (<i>Independent Variable</i>).....	28
4.6.2	Variabel Eksperimental (<i>Experimental Variable</i>).....	28
4.6.3	Variabel Tergantung (<i>Dependent Variable</i>).....	28
	cTnT	28
4.6.4	Variabel Kendali	28
4.7	Bahan dan Alat Penelitian	28
4.8	Definisi Operasional.....	30
4.9	Protokol Penelitian	30
4.9.1	Penelitian Tahap 1 : Isolasi AMSCs dari jaringan adiposa.....	30
4.9.2	Penelitian Tahap 2 : Karakterisasi <i>M-AMSCs</i> berupa identifikasi secara phenotype dengan CD105+, CD34+ dan CD45-	31
4.9.3	Penelitian Tahap 3 : Kultur AMSCs pada medium α -mem dan diferensiasi kardiomyosit	32
4.9.4	Penelitian Tahap 4 : Isolasi PRF	32
4.9.5	Penelitian Tahap 5 : Pemberian PRF pada kultur AMSCs <i>in vitro</i>	33
4.9.6	Penilaian Tahap 6 : Penilaian ekspresi marker diferensiasi kardiomyosit GATA-4 secara <i>Flow Cytometry</i> dan Troponin secara imunositokimia.....	33
4.10	Alur Penelitian	35
4.11	Pengumpulan, Pengolahan, dan Analisis Data	35
4.11.1	Pengumpulan Data	35
4.11.2	Pengolahan Data.....	36

4.11.3 Analisis Data	36
4.12 Ethical Clearance	36
BAB V HASIL PENELITIAN.....	37
5.1 Isolasi dan Kultur AMSCs.....	37
5.2 Karakterisasi Fenotipe AMSCs	37
5.3 Persiapan, isolasi dan pemberian PRF pada kelompok perlakuan	38
5.4 Penilaian ekspresi marker diferensiasi kardiomiosit GATA-4 secara <i>Flow Cytometry</i>	40
5.5 Penilaian ekspresi marker diferensiasi kardiomiosit Troponin secara Imunositokimia	44
BAB VI PEMBAHASAN.....	49
BAB VII KESIMPULAN DAN SARAN	54
7.1 Kesimpulan.....	54
7.2 Saran	54
DAFTAR PUSTAKA	55
LAMPIRAN	58

DAFTAR GAMBAR

Gambar 2.1 Isolasi dan Kultur Adypocyte Derived Mesenchymal Stem cells (AMSCs)	10
Gambar 2.2 Aplikasi MSC pada Regenerasi Kardiomiosit	11
Gambar 2.3 Diagram sekuensial proses pembentukan kardiomiosit dari sel pluripotent dan marker tipikal yang terekspresi dari setiap sekuensnya.....	14
Gambar 2.4 Produk platelet (<i>Platelet-rich hemoderivatives</i>)	15
Gambar 2.5 Alat sentrifus	16
Gambar 2.6 Putaran sentrifus pada PRF	16
Gambar 2.7 Flowchart Preparasi Platelet Rich Fibrin (PRF).....	17
Gambar 4.1 Desain Penelitian “Posttest Only Control Group Design”	25
Gambar 4.2 Diagram Alur Penelitian.....	35
Gambar 5.1 Kultur AMSCs.....	37
Gambar 5.2 <i>Flow cytometry</i> karakteristik AMSCs	38
Gambar 5.3 Tahap pembuatan PRF	39
Gambar 5.4 <i>Flow cytometry</i> dari kelompok medium α -MEM.....	41
Gambar 5.5 <i>Flow cytometry</i> dari kelompok medium diferensiasi	41
Gambar 5.6 <i>Flow cytometry</i> dari kelompok perlakuan.....	42
Gambar 5.7 Diagram batang <i>mean</i> rasio ekspresi GATA-4 dibandingkan dengan yang tidak terekspresi GATA-4 pada kelompok AMSCs.....	43
Gambar 5.8 Imunositokimia dari kelompok kontrol (-).....	45
Gambar 5.9 Imunositokimia dari kelompok kontrol (+).....	46
Gambar 5.10 Imunositokimia dari kelompok perlakuan.....	46
Gambar 5.11 Diagram batang rata-rata sel kardiomiosit melalui ekspresi troponin	47

DAFTAR TABEL

Tabel 2.1 Kriteria Identifikasi pada MSCs menurut ISCT	7
Tabel 2.2 Aksi biologi dari PRF	20
Tabel 4.1 Definisi Operasional	30
Tabel 5.1 Uji perbedaan T-test antara kelompok kontrol negatif, kontrol positif, dan perlakuan secara <i>Flow Cytometry</i>	44
Tabel 5.2 Uji perbedaan T-test antara kelompok kontrol negatif, kontrol positif, dan perlakuan secara imunositokimia	48

DAFTAR SINGKATAN

AMSCs	<i>Adipocyte-Derived Mesenchymal Stem Cell</i>
Ang-II	<i>Angiotensin-II</i>
ASCs	<i>Adipose stem cells</i>
Aza	<i>Azacytidine</i>
BMPs	<i>Bone Morphology Proteins</i>
BMSCs	<i>Bone Marrow-Derived Mesenchymal Stem Cells</i>
CD	<i>Cluster of Differentiation</i>
cTnT	<i>Cardiac Troponin T</i>
EGF	<i>Epidermal Growth Factor</i>
END-2 cells	<i>Endoderm-Like cells</i>
ESCs	<i>Embryonic Stem Cell</i>
FGFs	<i>Fibroblast Growth Factors</i>
IGF	<i>Insulin-Like Growth Factor</i>
ISCT	<i>International Society of Cellular Therapy</i>
miR	<i>Micro RNA</i>
MLC	<i>Myosin Light Chain</i>
MSCs	<i>Mesenchymal Stem Cells</i>
PBS	<i>Phosphate Buffer Saline</i>
PDGF	<i>Platelet-Derived Growth Factor</i>
PLA cells	<i>Processed Lipoaspirate Cells</i>
PRF	<i>Platelet Rich Fibrin</i>
PRP	<i>Platelet Rich Plasma</i>
SVF	<i>Stromal-Vascular Fraction</i>
TGF	<i>Transforming Growth Factor</i>
VEGF	<i>Vasoendothelial Growth Factor</i>
WHO	<i>World Health Organization</i>