Efek Pemberian Platelet-Rich Plasma terhadap Angiogenesis Adipose-Derived Mesenchymal Stem Cell Penderita Diabetes Melitus Tipe 2: Tinjauan In Vitro pada VEGF = The Effect of Platelet-Rich Plasma in Angiogenesis of Adipose-Derived Mesenchymal Stem Cell Diabetes Mellitus Type 2 Patient: In Vitro Review on VEGF

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Abstrak

Latar Belakang: Diabetes melitus (DM) tipe 2 adalah suatu penyakit metabolik yang kompleks dan kronis yang ditandai dengan gangguan angiogenesis. Inflamasi kronis derajat ringan dan stres oksidatif yang meningkat pada DM tipe 2 diketahui dapat menyebabkan gangguan fungsi biologis pada sel progenitor/sel punca vaskular, salah satunya adalah adipose-derived mesenchymal stem cell (ADSC). Sejumlah penelitian menunjukkan potensi vaskulogenik ADSC dan perannya pada regenerasi jaringan. Hingga saat ini aplikasi sel punca autologus pada penderita DM untuk menginisiasi vaskularisasi masih mengalami kendala. Platelet-rich plasma (PRP) diketahui kaya akan berbagai faktor pertumbuhan, termasuk VEGF, yang penting untuk proses angiogenesis. Tujuan: Penelitian ini bertujuan untuk menguji dan menganalisis efek pemberian PRP PMI terhadap proliferasi (jumlah sel stromal, nilai population doubling time (PDT), dan persentase sel hidup), diferensiasi (pembentukan koloni, ekspresi CD73, CD90, CD105, dan tiga lini diferensiasi), ekspresi mRNA VEGF dan VEGFR2, dan potensi angiogenik ADSC DM in vitro (sekresi VEGF dan pembentukan tubular kapiler). Metode: Terlebih dahulu, konsentrasi trombosit per µL dan kadar VEGF per 1x10³ trombosit pecah yang terkandung dalam PRP Palang Merah Indonesia (PMI) dibandingkan dengan PRP DM dan non-DM. Lalu, stromal vascular fraction (SVF) diisolasi dari jaringan lemak menggunakan metode enzimatik, dan SVF penderita DM tipe 2 (n= 15) dan non-DM (n= 10) dikultur dalam medium kontrol hingga didapat ADSC pasase 13 (P1P3). Proliferasi, diferensiasi, ekspresi mRNA VEGF dan VEGFR2, serta potensi angiogenik ADSC DM dan non-DM diukur dan dibandingkan. ADSC DM P3 kemudian dikultur dalam medium PRP PMI 5%, 10%, 15%, dan 20%, lalu proliferasi, diferensiasi, ekspresi mRNA VEGF dan VEGFR2 diukur dan dibandingkan dengan kontrol (FBS) untuk mendapatkan konsentrasi PRP optimum. ADSC DM P3 yang diprekondisikan dengan PRP optimum, dengan atau tanpa anti-VEGF (bevacizumab) 100 ng/mL, dan ADSC DM P3 kontrol dikultur, lalu sekresi VEGF dan pembentukan tubular kapiler pada Matrigel® diukur. Hasil: Pada penelitian ini tidak ditemukan perbedaan bermakna antara konsentrasi trombosit per µL PRP DM, non-DM, dan PMI (p= 0,22). Namun, PRP non-DM memiliki kadar VEGF per 1000 trombosit pecah lebih rendah bermakna (0,20 (0,040,35) fg) dibandingkan PRP DM (0,69 (0,211,17) fg), p= 0,03) dan PMI (1,84 (1,382,10) p= 0,01), dan tidak ada perbedaan bermakna antara PRP DM dan PRP PMI (p= 0,06). Jumlah sel stromal per gram lemak dan jumlah koloni sel stromal DM lebih rendah dari non-DM (86,35 (52,48106,76) x 10⁶ vs 158,93 (101,59185,94) x 10⁶, p= 0,01, dan 94 ± 14 koloni vs 31 ± 32 koloni, p= 0,004). Tidak terdapat perbedaan bermakna antara DM dan non-DM pada persentase sel stromal hidup (p= 0,24), ekspresi CD73 (p= 0,21), CD90 (p= 0,90), adipogenesis, kondrogenesis, osteogenesis, PDT P2 (p= 0,27), PDT P3 (p= 0,21), dan persentase sel hidup

ADSC P2 (p= 0,07), sedangkan ekspresi CD105 (64,41 (51,2073,38)% vs 91,40 (82,6295,47)%, p< 0,001) ADSC DM P1 dan persentase sel hidup (82,70 ± 8,07% vs 91,15 ± 3,77%, p= 0,04) ADSC DM P3 lebih rendah bermakna dibandingkan ADSC non-DM. Tidak ada penurunan yang bermakna pada ekspresi relatif mRNA VEGF (0,64 (0,301,08), p= 0,86) dan VEGFR2 DM (0,64 ± 0,56, p= 0,49) jika dibandingkan dengan ADSC non-DM. Rerata kadar VEGF dalam conditioned medium (CM) yang disekresikan oleh 1x10³ ADSC DM dan non-DM secara berturut-turut sebesar 0,74 pg/mL dan 0,62 pg/mL. ADSC DM yang diberi PRP optimum, yaitu 15% memiliki nilai PDT yang lebih rendah (2,33 ± 0,56 hari vs 5,04 ± 1,26 hari, p= 0,01) dan persentase sel hidup (95,53 ± 1,60% vs 78,95 ± 10,13%, p=0,01) yang lebih tinggi bermakna dibandingkan dengan kontrol. Terjadi peningkatan ekspresi CD105, mRNA VEGF, dan VEGFR2 ADSC DM yang diberi PRP 15% (secara berturut-turut 1,81 ± 0,73, p= 0,01; 5,27 ± 5,69, p= 0,23; dan 9,01 ± 11,59, p= 0,06) relatif terhadap kontrol. ADSC DM yang diberi PRP 15% dan ADSC DM kontrol secara berturut-turut mensekresikan VEGF rerata sebanyak 0,57 pg/mL dan 1,67 pg/mL per 1x10³ sel hidup. Jumlah tubular kapiler in vitro ADSC DM yang diberi PRP meningkat pada jam ke-24 jika dibandingkan dengan kontrol dan tidak berbeda bermakna dengan ADSC non-DM, namun membutuhkan waktu lebih panjang, serta tidak berbeda bermakna dengan ADSC DM yang diberi PRP dan anti-VEGF (p=0,78). Kesimpulan: ADSC DM terbukti mengalami kerusakan selular yang dicirikan dengan penurunan proliferasi, diferensiasi, ekspresi mRNA VEGF dan VEGFR2, serta potensi angiogeniknya. Pemberian PRP 15% (VEGF 98,00 pg/mL) dapat memperbaiki kerusakan tersebut melalui efek sinergis yang dihasilkan oleh VEGF dan faktor pertumbuhan lainnya yang terdapat dalam PRP. <hr /> Background: Type II diabetes mellitus (DM type 2) is a chronic and complex metabolic disease identified by impaired angiogenesis. Low grade chronic inflammation and increasing oxidative stress in DM type 2 decrease the biological functions of progenitor/stem cells, including adipose-derived stem cells (ADSC). ADSC plays significant roles in angiogenesis and tissue regeneration. Some studies have shown the vasculogenic potency of ADSC and its role in tissue regeneration. To date, autologous cell application in DM patients to initiate vascularization is hindered. Platelet-rich plasma (PRP) is widely known to contain generous amount of growth factors including VEGF with significant role in angiogenesis. Objective: This study aimed to investigate and analyze the effect of PRP preconditioning to the proliferation (stromal cell number, population doubling time (PDT) and percentage of viable cells), differentiation (colony formation, CD73, CD90, CD105 expressions, three lineage of differentiation), expression of mRNA VEGF and VEGFR2, as well as in vitro angiogenic potency of ADSC DM (VEGF secretion and capillary tube formation). Methods: Initially, platelet concentration per µL and VEGF per 1x10³ lysed platelet contained in Palang Merah Indonesia (PMI) PRP was compared to DM and non-DM PRP. Subsequently, stromal vascular fraction (SVF) from 15 DM and 10 non-DM donors was enzymatically isolated from adipose tissue, and cultured in control media to generate passage 13 (P1P3) ADSC. Proliferation, differentiation, mRNA VEGF and VEGFR2 expression, as well as angiogenic potency of DM ADSC in vitro were measured and compared to non-DM control. P3 DM ADSC was then cultured in media contained 5%, 10%, 15%, and 20% PMI PRP, and proliferation, differentiation, mRNA VEGF and VEGFR2 expression were measured and compared to FBS control to determine optimum PMI PRP concentration. P3 DM ADSC preconditioned with optimum PMI PRP, with or without anti-VEGF (bevacizumab) 100 ng/mL, and control DM ADSC were cultured, and VEGF secretion was measured, as well as capillary tube formation on Matrigel®. Results:

In this study no significant differences were observed between platelet concentration per µL DM, non-DM, and PMI PRP (p= 0.22). However, non-DM PRP contained significantly lower VEGF per 1000 lysed platelets (0.20 (0.040.35) fg) compared to DM (0.69 (0.211.17) fg, p= 0.03) and PMI PRP (1.84 (1.382.10), p= 0.01), with no significant difference between DM and PMI PRP (p=0.06). The number of viable stromal cells per gram adipose tissue and collonies generated from DM SVF were significantly lower than non-DM (86.35 (52.48106.76) x 10⁶ vs 158.93 (101.59185.94) x 10⁶, p= 0.01 and 94 ± 14 collonies vs 31 ± 32 collonies, p= 0.004). Non-significant differences were also observed in the percentage of viable stromal cells (p= 0.24), expression of CD73 (p= 0.21), CD90 (p= 0.90), adipogenesis, chondrogenesis, osteogenesis, P2 and P3 PDT (p= 0.27 and 0.21, respectively), and the percentage of viable P2 ADSC (p= 0.07), but the expression of CD105 of P1 DM ADSC (64.41 (51.2073.38)% vs 91.40 (82.6295.47)%, p< 0.001) and the percentage of viable P3 ADSC (82.70 ± 8.07% vs 91.15 ± 3.77%, p= 0.04) were significantly lower than non-DM ADSC. The reduction of mRNA VEGF and VEGFR2 relative expression of P3 DM ADSC (0.64 (0.301.08) p= 0.86 and 0.64 ± 0.56, p= 0.49, respectively) were unsignificant compared to non-DM ADSC. Mean of VEGF level normalized to 1x10³viable cells in the conditioned medium (CM) of DM and non-DM ADSC were 0.74 pg/mL and 0.62 pg/mL, respectively. Optimum 15% PRP-preconditioned DM ADSC had significantly lower PDT value (2.33 ± 0.56 days vs 5.04 ± 1.26 days, p=0.01) and higher percentage of viable cells compared to control (95.53 ± 1.60% vs 78.95 ± 10.13%, p=0.01). The increase of relative expression of CD105, mRNA VEGF and VEGFR2 (1.81 ± 0.73, p= 0.01; 5.27 ± 5.69, p= 0.23; and 9.01 ± 11.59, p= 0.06, respectively) were unsignificant in DM ADSC compared to non-DM. Optimum 15% PRP-preconditioned DM ADSC and control secreted VEGF in CM as much as 0.57 pg/mL and 1.67 pg/mL per 1x10³ viable cells in average. PRP-preconditioning improved the capillary tube formation in DM ADSC, but the process was longer compared to control, and unsignificant to non-DM ADSC and PRP-preconditioned DM ADSC with anti-VEGF (p=0.78). Conclusions: Cellular damage in DM ADSC was idientified by a reduction of proliferation, differentiation. mRNA VEGF and VEGFR2 expression, and angiogenic potency. Preconditioning DM ADSC with 15% PRP (VEGF 98.00 pg/mL) improved the cellular damage with synergitic effect of VEGF and other growth factors contained in PRP.