Pengembangan Terapi Infark Miokard dengan Injeksi Hidrogel Transepikardial dan Implantasi Perancah Patch Membran Amnion yang Dideselularisasi di Epikardial menggunakan Amniotic Epithelial Cells dengan dan Tanpa Ko-kultur Kardiomiosit Secara In Vivo = Development of Myocardial Infarction Treatment with Transepicardial Hydrogel Injection and Epicardial Decellularized Amniotic Membrane Scaffold Patch Implantation using Amniotic Epithelial Cells and Cardiomyocyte Co-Culture

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Abstrak

Infark miokard menyebabkan kematian kardiomiosit dan remodeling jantung pada situasi patologis. Pascainfark jantung tidak mampu mengatasi kehilangan kardiomiosit meskipun telah dilakukan rekanalisasi atau revaskularisasi. Oleh karena itu, diperlukan metode untuk mengembalikan fungsi jantung. Sel punca dapat memperbaharui diri dan berdiferensiasi menjadi berbagai tipe sel namun kesintasannya pada pasien masih rendah. Untuk meningkatkan retensi dan regenerasi sel punca di miokardium dapat digunakan perancah/scaffold dan sistem ko-kultur, namun belum ada penelitian tentang hal tersebut. Penelitian ini bertujuan mengembangkan terapi infark menggunakan injeksi hidrogel transepikardial dan implantasi di epikardial perancah patch membran amnion yang dideselularisasi menggunakan amniotic epithelial cells (AEC) dengan ko-kultur kardiomiosit. Penelitian ini menggunakan post-test only control group design yang dilakukan di Institut Pertanian Bogor dan Fakultas Kedokteran Universitas Indonesia dari Juli 2021–Oktober 2022. Subjek penelitian adalah 15 babi Sus scrofa domesticus usia 2-3 bulan dibagi tiga kelompok: pAEC, pAEC + kardiomiosit, kontrol positif, dan 1 babi sebagai kontrol negatif. Torakotomi dilakukan untuk membuat model infark dengan ligasi arteri proximal branch to left ventricle (PLV) dilanjutkan implantasi pAEC dengan atau tanpa ko-kultur kardiomiosit pada kelompok terapi, kemudian diobservasi selama 6–8 minggu. Luas infark diukur dengan late gadolinium enhancement MRI; remodeling ventrikel kiri dengan ekokardiografi untuk menilai kontraktilitas, fibrosis dengan IHK, kardiomiogenesis dan regulasi apoptosis dengan RT-PCR, angiogenesis dinilai dengan IHK, dan fraksi ejeksi dinilai dengan ekokardiografi. Luas infark menurun pada kedua kelompok terapi (2,5 [2,00–3,00]% dan 3,60 ± 1,34% vs 9,50 ± 1,91%). Pewarnaan HE dan Masson trichrome menunjukkan berkurangnya proses fibrosis pada kedua kelompok, dikonfirmasi dengan hiperekspresi kolagen1 yang padat dan kaku pada kontrol positif dibandingkan kedua kelompok terapi yang memiliki ekspresi kolagen3 lebih dominan. Ekspresi -smooth muscle actin pada kedua kelompok tampak tersebar menunjukkan penurunan fibrosis dan kontrol positif menunjukkan peningkatan fibrosis. Peningkatan kardiomiogenesis pada kedua kelompok dikonfirmasi dengan peningkatan ekspresi gen cardiac troponin T, gen myosin heavy chain, gen Nkx.2.5, gen c-Kit, dan penanda otot fungsional -actinin. Penurunan apoptosis dikonfirmasi dengan penurunan ekspresi gen modulator apoptosis p21 dan ekspresi gen p53 yang berarti diferensiasi sel punca tidak bersifat tumorigenik. Regulasi apoptosis melalui ekspresi kaspase-9 tidak berbeda bermakna. Peningkatan angiogenesis dikonfirmasi dengan peningkatan ekspresi von Willebrand Factor dan ekspresi -smooth muscle actin yang tersebar. Ekokardiografi menunjukkan perbaikan regional wall motion abnormality lebih banyak pada kelompok terapi daripada

kontrol positif dan fraksi ejeksi tidak berbeda bermakna antar kelompok. Disimpulkan kombinasi injeksi hidrogel transepikardial dan implantasi di epikardial perancah patch membran amnion yang dideselularisasi dengan ko-kultur AEC dan kardiomiosit dapat mengurangi luas infark dan remodelling ventrikel kiri, serta meningkatkan angiogenesis pada babi model infark.

.....Myocardial infarction induces cardiomyocyte death and remodelling a pathological condition. The post-infarct heart is unable to deal with cardiomyocyte loss despite recanalization or revascularization. Therefore, a procedure is required to restore cardiac function. Stem cells can self-renew and specialize into multiple cell types however the survival of stem cells in patients is still poor. To promote the retention and regeneration of stem cells in the myocardium, scaffolds and co-culture systems may be applicated, although there are no study findings on this issue. This study aimed to develop myocardial infarction therapy using transepicardial hydrogel injection and epicardial decellularized amniotic membrane scaffold patch implantation using amniotic epithelial cells (AEC) with cardiomyocyte co-culture. This study used a post-test-only control group design performed at the IPB University and the Faculty of Medicine, Universitas Indonesia, from July 2021 to October 2022. The study subjects were 15 Sus scrofa domesticus pigs aged 2-3 months placed into three groups: pAEC, pAEC + cardiomyocytes, positive control, and 1 pig as a negative control. Thoracotomy was conducted to create an infarct model with the proximal branch to left ventricle (PLV) artery occlusion followed by pAEC implantation with or without cardiomyocyte co-culture in the therapy group, then evaluated for 6–8 weeks. Infarct size was determined by late gadolinium enhancement MRI, left ventricular remodeling by echocardiography to evaluate contractility, fibrosis by IHC, cardiomyogenesis and regulation of apoptosis by RT-PCR, angiogenesis was assessed by IHC, and ejection fraction by echocardiography. Infarct size reduced in both therapy groups (2,5 [2,00–3,00]% and 3,60 ± 1,34% vs 9,50 ± 1,91%). HE and Masson trichrome staining demonstrated decreased fibrosis in both groups, confirmed by hyperexpression of dense and stiff collagen 1 in the positive control compared to the two therapy groups with more dominant collagen 3 expressions. The -smooth muscle actin expression in both groups seemed to be scattered suggesting reduced fibrosis while the positive control showed increased fibrosis. Increased cardiomyogenesis in both groups was confirmed by increased expression of the cardiac troponin T gene, the myosin heavy chain gene, the Nkx.2.5 gene, the c-Kit gene, and the functional muscle marker -actinin. The reduction in apoptosis has been confirmed by lower expression of the p21 apoptosis modulator gene and p53 gene expression, which suggests that stem cell differentiation is not tumorigenic. The control of apoptosis by caspase-9 expression was not significantly different. Increased angiogenesis was verified by increased von Willebrand Factor expression and scattered expression of -smooth muscle actin. Echocardiography showed greater improvement in regional wall motion abnormalities in the therapy groups than in the positive control, and the ejection fraction was not significantly different between groups. It was concluded that the combination of transepicardial hydrogel injection and epicardial decellularized amniotic membrane scaffold patch implantation using AEC with cardiomyocyte co-culture could reduce infarct size and left ventricular remodeling, as well as increase angiogenesis in infarct model pigs.