Diferensiasi Sel Punca Mesenkimal menjadi Pacemaker-like Cells dengan Transfeksi Tbx3 dan Pemberian Small Molecule dalam Upaya Inovasi Terapi Bradiaritmia = Differentiation of Mesenchymal Stem Cells into Pacemaker-like Cells by Tbx3 Transfection and Addition of Small Molecules in Innovation of Bradyarrhythmia Therapy

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Abstrak

Bradiaritmia seperti total AV blok dan disfungsi nodus SA (NSA) memerlukan alat pacu jantung permanen (APJP), namun APJP memiliki potensi komplikasi akut dan jangka panjang. Sel punca mesenkimal (SPM) dapat mengatasi kerusakan nodus sinoatrial (NSA) dan nodus atrioventrikel (NAV), namun transplantasi SPM secara langsung pada hewan model memiliki angka keberhasilan rendah. Untuk mengatasi masalah tersebut, diperlukan sel punca yang telah terdiferensiasi menjadi pacemaker-like cell seperti sel NSA atau NVA sehingga siap ditransplantasikan ke area disfungsi atau blokade. Penelitian ini bertujuan untuk mendiferensiasi SPM asal jaringan adiposa (SPMA) menjadi pacemaker-like cells. Penelitian ini menggunakan desain eksperimental dan dilakukan pada bulan Februari 2022 sampai Maret 2023 di IMERI-FKUI. Terdapat 5 kelompok penelitian, yaitu: 1) kultur SPMA tanpa intervensi (kontrol), 2) kultur SPMA yang didiferensiasi menjadi kardiomiosit, 3) kultur SPMA yang didiferensiasi menjadi kardiomiosit dan ditransfeksi gen Tbx3 (TBX), 4) kultur SPMA yang didiferensiasi menjadi kardiomiosit dan diberikan small molecules SB431542, serta 5) kultur SPMA yang didiferensiasi menjadi kardiomiosit, ditransfeksi gen Tbx3 dan diberikan small molecule SB431542 (TBX+SM). Pemeriksaan ekspresi gen penanda pacemaker-like cells (Tbx3, Cx30, Cx40, Cx43, HCN1, HCN3, HCN4, dan KCNN4) menggunakan metode qRT-PCR pada kelompok TBX, SM, dan TBX+SM menunjukkan peningkatan ekspresi gen Tbx3, Cx30, HCN1, HCN3, HCN4 dan KCNN4 yang berbeda bermakna terhadap kelompok kardiomiosit (p 0,001) sedangkan penurunan ekspresi gen Cx40 dan Cx43 berbeda bermakna dibandingkan kelompok kardiomiosit (p < 0,001). Eskpresi protein Tbx3 dan Cx30 menggunakan ELISA pada kelompok TBX, SM, dan TBX+SM berbeda bermakna terhadap kelompok kardiomiosit (p 0,001). Gambaran ekspresi protein Tbx3 dan Cx30 menggunakan metode imunofluoresensi menunjukkan pendaran positif pada kelompok TBX, SM, dan TBX+SM. Morfologi elektrofisiologis dengan patch clamp menunjukkan gambaran potensial aksi khas pacemaker-like cells pada kelompok TBX, SM, dan TBX+SM dinilai dari rasio action potential duration90/action potential duration50 (APD90/APD50). Disimpulkan transfeksi gen Tbx3, pemberian small molecules SB431542, dan kombinasi keduanya mampu mendiferensiasikan SPMA menjadi pacemaker-like cells.

.....Bradyarrhythmias, such as total AV block and SA node dysfunction, require a permanent pacemaker (PPM); however, PPM has the potential for acute and long-term complications. Mesenchymal stem cells (MSC’s) can repair damaged sinoatrial (SAN) and atrioventricular (AVN) nodes; however, transplantation of MSCs into animal models has a low success rate. To overcome this problem, it is necessary to have stem cells that differentiate into pacemaker-like cells, such as SAN or AVN cells, so that they can be transplanted to areas of dysfunction or blockage. This study aimed to differentiate MSC’s from adipose tissue (AMSC) into pacemaker-like cells. This study used an experimental design and was conducted from February 2023 to

March 2023 at the IMERI-FKUI. There were five study groups, namely:1) AMSC cultures without intervention (control), 2) AMSC cultures that differentiated into cardiomyocytes, 3) AMSC cultures that differentiated into cardiomyocytes and transfected with the Tbx3 (TBX) gene, 4) AMSC cultures that differentiated into cardiomyocytes and administered SB431542, and 5) AMSC culture, which differentiated into cardiomyocytes, were transfected with the Tbx3 gene and administered SB431542 (TBX+SM). RT-qPCR expression of pacemaker-like cell marker genes (Tbx3, Cx30, Cx40, Cx43, HCN1, HCN3, HCN4, and KCNN4) in the TBX, SM, and TBX+SM groups showed increased expression of Tbx3, Cx30, and HCN1., HCN3, HCN4, and KCNN4, which differed significantly in the cardiomyocyte group (p 0.001), whereas the decrease in Cx40 and Cx43 gene expression was significantly different compared to that in the cardiomyocyte group ($p < 0.001$). ELISA of Tbx3 and Cx30 protein expression in the TBX, SM, and TBX+SM groups was significantly different from that in the cardiomyocyte group (p 0.001). Immunofluorescence analysis of Tbx3 and Cx30 protein expression showed a positive correlation in the TBX, SM, and TBX+SM groups. The electrophysiological morphology with the patch clamp showed a typical action potential picture of pacemaker-like cells in the TBX, SM, and TBX+SM groups, as assessed by the ratio of action potential duration90/action potential duration50 (APD90/APD50). It was concluded that transfection of the Tbx3 gene, administration of the small molecule SB431542, and a combination of both could differentiate SPMA into pacemaker-like cells.