Perbandingan dan korelasi ekspresi Tenascin C dengan jumlah dan diameter sel otot jantung tiga kelompok usia tikus = Comparison and correlation between Tenascin C expression and cardiomyocites number and diameter in three age groups rat cardiac

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Abstrak

Latar Belakang: Penemuan sel stem jantung (CSC, cardrkzc stem cells) membuktikan jantung sebagai organ dengan pergantian sel-sel parenkim dan non-parenkim di bawah pengaturan kompartemen sel stem. Kemampuan regenerasi jantung berkurang dengan bertambahnya usia. Penyebab penuaan sel stem jantung adalah perubahan pada lingkungan mikro (niche) jantung yang mempengaruhi keberlangsungan hidup sel stem jantung. Tenascin C adalah molekul di niche jantung yang berperan dalam remodeling jaringan jantung dan angiogenesis, dna komponen peuting dalam regenerasi jantung. Penelitian ini bertujuan untuk mengetahui jumlah dan diameter sel otot jantung di jantung tikus yang berbeda usia, menilai ekspresi Tenascin C dan mengetahui hubungan antara ekspresi tenascin C dan perubahan morfometri sel otot jantung.

Metode: Desain penelitian ini adalah komparatif potong lintang dengan 6 tikus neonatus (usia 1-4 hari), 9 tikus dewasa muda (usia 3-4 bulan) dan 9 tikus dewasa (usia 12-16 bulan). Proses pembuatan sediaan mikroskopik dilanjutkan dengan pewarnaan HE dan imunohistokimia Tenascin C (sc-9871, sc~2023). Mikrofografi jamung (HE) dipilih 2 Ipb atrium dan 2 Ipb ventrikel. Hasil mikrofotograf dimasukkan dalam format jpeg dan dianalisis dengan Digimizer Image Analyzer. J umlah sel otot jantung dihitung per Ipb dengan tagging system dan diameter sel otot jantung diukur berdzsar unit kalibrasi skala mikrometer. Milcrofotograf tenascin C Iewat software DP2BSW dalam format tifl Dihitung 100 sel otot jantung atrium dan 100 sol ventrikel untuk musing-masing subyek. Imunoreaktivitas tenascin C di sel otot jantung dinyatakan lokasi ekqaresi dan skor intensitas. Lokasi ekspresi adalah positif intra sel, ekstra seL kombinasi keduanya dan negatifl [ntensitas pewarnaan tenascin C diberi skor 1 (lemah) sampai 3 (lcuat). Analisis statistik menggunakan SPSS 13.

Hasil : Jumlah sel otot jantung per Ipb terbesar di kelompok neonatus (Atrium=73.4:l=4.8'7; Ventrikel= l52.5:1:3.6) dan paling sedikit di kelompok dewasa (Atrium= 26:I:1.5; Ventrikel= 43.7:1:2.8). Diameter sel otot jantung terkecil di kelompok neonatus (Atrium= 6.lprni0.23; Ventrikel=-° 7.39pmi0.3) dan paling besar di kelompok dewasa (Atrium°-= l7.42pmi0.42; Ventrikel== 23.44|1m=1=0.74). Ekspresi tenascin C ditemukan pada jantung tikus neonatus, dewasa ruuda dan dewasa. Pola ekspresi tenascin C yang sering ditemukan di kelompok neonatus adalah pola kombinasi (Atrium= 43.l7i9.4, Ventrikel= 56.83=l=8.5) dan pola intra sel (Atrium-= 41.33=+=13.4; Ventrikel= 33 .67:|:6.7). Pola ekspresi tenascin C ekstra se! lebih sering ditemukan di kelompok dewasa muda (Atrium= ll.56t3.2; Ventrikel= l2.ll=b7.4) dan dew:-lm (Atrium= 9.22=l:3.5; Ventrikel= 11.67:E3.9) dibandingkan kelompok neonatus (Atrium= 3.33:I=1.3; Ventrikel= 2.5¢l.4). Ekspresi tenascin C negatif paling sering ditemukan di ventrikel jantung dewasa muda (74.44t8 2) dan dewasa (67 .33=\:?7 .6) . Intensitas pewamaan tenascin C kuat (skor 3) paling sering ditemukan di kelempok neonatus (Atrium= 42.83=1=l3.6; Venti-ikel= 59.33=1=9). Skor I paling sering ditemukan di ventrikel jantung kelompok dewasa (16.1 l=|=5.3). Dari analisis korelasi bivariat Pearson

ditemukan korelasi positif yang bermakna antara pola ekspresi tenascin C kombinasi di atrium dengan jumlah sel ototjantung atrium (p==0.0l6); pola ekspresi tenasein C intra sel di ventrikel dengan jumlah sel otot jantung ventrikel (p=0.0l) dan pola ekspresi kombinasi di ventrikel dengan jumlah (p=0.00) dan diameter sel otot jantung vcntrikel (p=0.026). Ditemukan pula korelasi positif yang bermakna aniara sl-cor 3 intensitas pewarnaan tenascin C di atrium dengan jumlah sel otot jamung atrium (p=0.035); skor 3 di ventrikel dengan jumlah sei otot jantung ventrikel (p=0.00). Korelasi negatif yang bermakna ditemukan antara skor 3 di ventrikel dengan diameter sel otot jantung ventrikel (p=0.0~0l).
Kesimpulan : Semakin bertambah usia jantung, jumlah sel/Ipb semakin berkurang dan diameter semakin besar. Gambaran ini menandalcan teajadinya hipertrofi sel otot jantung. Ekspresi tenascin C ditemukan di jantung neonatus, dewasa muda dan dewasa. Semakin bertambah usia jantung terjadi penurunan jumlah sel otot jamung yang positif mengekspresikan tcnascin C dan berkurangnya intensitas pewarman tenascin C. Di atrium dan ventrikel jamung, semakin banyak jumlah sel otot dengan pola ekspresi tenascin C kombinasi maka semakin banyak jumlah sel otot jantung. Di ventrikel, pola ekspresi kombinasi juga berkorelasi positif dengan diameter se] otot jantung. Semakin tinggi jumlah sel dengan skor intensitas 3 make jumlah sel Otot jantung semakin banyak dan diameter sel otot jantung yang kecil.
<hr><i>Background: Discovery of Cardiac Stem Cells (CSC) showed the heart as renewable organ with parenchymal and non-parenchymal cells turnover governed by stem cells compartments. Cardiac regenerative ability decreases with advancing age. The cause of CSC?s aging is the changes in cardiac microenvironment (niche) that surrounds CSC. Tenascin C is a major glycoprotein in cardiac niche that plays a vital role in cardiac remodelling and angiogenesis, two main components of cardiac regeneration. This study aims to compare immunoreactivity of tenascin C, cardiomyocites number and diameter in three age groups rat cardiac and determine the correlation between tenascin C immunoreactivity and cardiomyocite?s motphometric changes.
Methods: Design of this study is comparative cross sectional with 6 neonate rats (age I-4 days), 9 young adult rats (age 3-4 months), and 9 adult rats (age 12-16 months). The subjects underwent intravital lixation and cardiac organ was removed. Microscopic specimens were made and stained with hematoxylin-Eosin and tenascin C immunohistochemistry (sc-9871, sc-2023). From cardiac microphotograph (HE stained) two high power field (hpf) was selected for atrium and two hpf for ventricle. Microphotographs was transferred into digital format (jpeg) and analysed with Digimizer Image Analyzer. Cardiomyocite number was determined using tagging system and measurement of cardiomyocite diameter was calibrated with micrometre scale using Digimiaer Image Analyzer. immunohistochemistry results were documented with DPZBSW as tnicrophotographs in digital format (tiff). 100 catrliomyocites in the atrium and in the ventricle fiom each subject was analysed. Immunoreactivity of tenascin C was classified based on expression paltem and staining intensity. The expression pattern was positive intra cellular, positive extra cellular, positive combination (both intra and extra cellular) and negative. Staining intensity was scored I (weak) to 3 (strong). Statistical analysis was performed with SPSS I3.
Result : The most abundant cardiomyocte number per high power fielf (hpf) was found in neonate cardiac (Atrium= 73.4=b4.8'7; Ventrikel= l52.5:l:3.6) and the least abundant was in adult cardiac(Atrium= 26=l:l.5; Ventrikel= 43.7=E2.8). Cardiomyocite diameter was smallest in neonate cardiac (Atrium= 6.1 um=h0.28; Ventrikel= 7.39um:I=0.3) and largest in adult group (Atrium= l7.42um:1:0.42; Ventrikel= 23.44|.un:l:0.74). Tenascin C immunoreactivity was found in neonate, adolescence and adult cardiac. Tenascin C expression pattern most frequently found in neonate cardiac was positive combination (Atrium= 43.l7:1:9.4, Ventrikel=

56.83=l:8.5) and positive intra cellular (Atrium= 4l.33il3.4; Ventrikel= 33.67=l:6.'7). Tenascin C positive extra cellular was commonly found in young adult cardiac (Atrium= 1l.56=l=3.2; Ventrikel= 12.l 1174) and adult cardiac (Atrium= 9.22d:3.5; Ventrikel= 11.671-3.9). Negative tenascin C was more ti-equently found in young adult ventricle (74.44=i=8.2) and adult cardiac (67.33:l:'7.6). High score for tenascin C staining intensity (score 3) was iiequently found in neonate cardiac (Atrium= 42.83=kl3.6; Ventrikel= 59.33d=9). Score l was iiequently found in adult ventricle (16.1l:l:5.3). Pearson bivariate correlation revealed significant correlation between positive combination tenascin C pattern in the atrium with atrial cardiomyocites number(p=0.0l6); positive intra cellular tenascin C pattem in the ventricle with ventricular cardiomyocitcs number (p=0.0l) and positive combination in the ventricle with ventricular cardiomyocites number (p=0.00) and diameter (p=0.026). Significant correlation was also found between score 3 in the atrium with atrial cardiomyocites number (p=0.035); score 3 in the ventricle with ventricular cardiomyocites number (p=0.00). Negative correlation was found signiiicant between score 3 in the ventricle with ventricular cardiomyocitcs diameter (p=0.00l).

Conclusions : With advancing age, cardiomyocte number per hpf decreases while the diameter increases. This resembles hypertrophy of cardiomyocite. Tenascin C immunoreactivity was found in neonate, adolescence and adult mrdiac tissue. With advancing age, we found reduced number of cardiomyocites expressing tenascin C and decreased staining intensity. In cardiac atrium and ventricle, increased number of positive combination tenascin C expression showed increased cardiomyocites number. In ventricle, increased number of positive combination showed increased cardiomyocitec diameter. Increased number of cardiomyocites with score 3 tenascin C staining intensity showed higher cardiomyocites number and smaller diameter.</i>