

Efek intermittent dan prolonged fasting terhadap ekspansi, proliferasi, dan diferensiasi sel punca mesenkimal darah perifer dan sumsum tulang pada kelinci New Zealand White = Effect of intermittent and prolonged fasting on expansion, proliferation, and differentiation of mesenchymal stem cell from peripheral blood and bone marrow on New Zealand White Rabbit

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Abstrak

Isolasi sel punca mesenkimal (SPM) dari darah perifer (DP) menutupi kekurangan yang ditemukan pada isolasi dari sumsum tulang (ST). Jumlah darah yang banyak dapat diperoleh dari sirkulasi perifer dan teknik pengambilannya lebih tidak traumatik dibandingkan pengambilan dari sumsum tulang. Namun, jumlahnya sedikit di dalam darah. Diperlukan suatu kondisi untuk meningkatkan hasil isolasi dari darah perifer. Restriksi kalori meningkatkan kemampuan self-renewal dari sel punca intestinal, sel punca otot dan regenerasi saraf, menjaga kemampuan regenerasi jangka panjang pada sel punca hematopoetik. Belum terdapat penelitian yang mempelajari efek intermittent atau prolonged fasting pada SPM darah perifer dan sumsum tulang, maka diperlukan penelitian untuk mempelajari efek fasting terhadap kemampuan proliferasi dan diferensiasi SPM. Penelitian ini menggunakan kelinci (n=27) yang dibagi menjadi tiga kelompok; setiap kelompok terdiri dari 9 kelinci. Kelompok pertama sebagai kontrol diberikan makan dan minum ad lib. Kelompok kedua mendapat perlakuan intermittent fasting (7 siklus), dan kelompok ketiga mendapat perlakuan prolonged fasting (4 siklus). Sampel diambil dari darah perifer dan sumsum tulang femur. Dilakukan isolasi kultur untuk menilai kemampuan proliferasi (waktu konfluensi dan jumlah sel) dan diferensiasi (kualitatif dan kuantifikasi) dari masing-masing kelompok sampel. Sel punca mesenkimal pada ketiga kelompok penelitian mampu diisolasi, berproliferasi dan berdiferensiasi menjadi osteoblas. Persentase keberhasilan kultur primer dari kelompok kontrol: DP 14.28%, dan ST 28.57%; kelompok IF: DP 44.44% dan ST 33.33%; dan kelompok PF: DP 55.55%, dan ST 44.44%. Rerata waktu konfluensi kelompok kontrol: DP 17 hari dan ST 31 hari; kelompok IF: DP 15 hari dan ST 26 hari; dan kelompok PF: DP 15.6 hari dan ST 20 hari (DP p=0.592, dan ST p=0.408). Rerata jumlah sel konfluensi kelompok kontrol: DP 108 x10³/mL dan ST 274 x10³/mL; kelompok IF: DP 182 x10³/mL dan ST 115.3 x10³/mL ; dan kelompok PF: DP 65.6 x10³/mL dan 139 x10³/mL ST (DP p=0.282 dan ST p=0.502). Rerata kuantifikasi optik densitometri pada diferensiasi osteoblas kelompok kontrol: DP 0.154 OD dan ST 0.169 OD; kelompok IF: DP 0.240 OD dan ST 0.207 OD; dan, kelompok PF: DP 0.157 OD dan ST 0.167 OD (DP p=0.044 dan ST p=0.074). Uji posthoc kuantifikasi optik densitometri diferensiasi osteoblas didapatkan perbedaan bermakna pada kelompok IF DP (p=0.046). Perlakuan intermittent dan prolonged fasting memiliki efek dalam meningkatkan ekspansi SPM ke darah perifer. Kuantifikasi diferensiasi osteoblas SPM-DP perlakuan IF lebih tinggi dibandingkan kontrol. Diharapkan ada penelitian lanjutan yang mengevaluasi efek intermittent fasting pada sampel darah perifer manusia terhadap kemampuan SPM dalam hal ekspansi, proliferasi dan diferensiasi menjadi osteoblas.

.....Isolation of mesenchymal stem cells (MSC) from peripheral blood (PB) was considered giving more advantages compared to isolation from bone marrow (BM). Large amounts of blood can be taken from

peripheral circulation by less invasive extraction technique than BM. However, MSC isolated from PB can only be achieved in a small amount. Some conditioning of the subjects are needed in order to improve the isolation products from PB. Calorie restriction increases the self-renewal ability of intestinal stem cells, muscle stem cells and nerve regeneration, and maintain the long-term regeneration ability of hematopoietic stem cells. There has not been any studies that explore the effects of intermittent or prolonged fasting on MSC of PB and BM. The aim of this study is investigating the effect of fasting on the ability of MSC proliferation and differentiation. This study used rabbits ($n = 27$) which were divided into three groups; each group consists of 9 rabbits. The first group as a control was given food and drink ad lib. The second group received intermittent fasting (7 cycles), and the third group received prolonged fasting (4 cycles). Samples were taken from the peripheral blood and femoral bone marrow. Culture isolation was performed to assess the proliferation (confluency time and cells number) and differentiation (qualitative and quantitative) abilities of each sample group. Mesenchymal stem cells in all groups were able to be isolated, proliferate and differentiate to osteoblast. The successful rate of primary culture from control group: PB 14.28% and BM 28.57%; IF group: PB 44.44% and BM 33.33%; and PF group: PB 55.55% and BM 44.44%. The mean of confluence time from control group: PB 17 days and BM 31 days; IF group: PB 15 days and BM 26 days; and PF group: DP 15.6 days and ST 20 days (PB $p=0.592$, and BM $p=0.408$). The mean of confluence cells number: PB $108 \times 10^3/\text{mL}$ and BM $274 \times 10^3/\text{mL}$; IF group: PB $182 \times 10^3/\text{mL}$ and BM $115.3 \times 10^3/\text{mL}$; and PF group: PB $65.6 \times 10^3/\text{mL}$ and $139 \times 10^3/\text{mL}$ ST (PB $p=0.282$ and BM $p=0.502$). The mean of optical densitometry quantification from osteoblast differentiation in control group: : PB 0.154 OD and BM 0.169 OD; IF group: PB 0.240 OD and BM 0.207 OD; and, PF group: PB 0.157 OD and BM 0.167 OD (PB $p=0.044$ dan BM $p=0.074$). Posthoc analysis from optical densitometry quantification of osteoblast differentiation showed significant difference on IF PB group ($p=0.046$). Intermittent and prolonged fasting treatment gave increasing effect of MSC expansion into peripheral blood. MSC-PB osteoblast differentiation quantification was higher in IF treatment compared to control. It is hoped that further studies will evaluate the effect of intermittent fasting on human peripheral blood samples in the ability of SPM in terms of expansion, proliferation and differentiation into osteoblasts. We suggest that there will be further studies conducted to evaluate the effect of intermittent fasting on the ability of MSC's expansion, proliferation, and differentiation into osteoblasts from human peripheral blood samples.