ABSTRAK

Latar Belakang. Hasil uji klinis senyawa neuroproteksi pada kasus iskemia otak dengan diabetes tidak sesuai dengan hasil uji pada hewan coba. Hal ini disebabkan karena adanya variasi teknik BCCAO pada tikus model iskemia, penyakit penyerta tidak ada atau tidak sesuai dan kuantifikasi pada hewan coba menggunakan metode yang bias. Berdasarkan hal tersebut maka perlu pengembangan hewan model iskemia otak global pada tikus model diabetes dengan memperhatikan variasi model iskemia otak (durasi iskemia, durasi reperfusi, durasi hiperglikemia) dengan parameter target volume iskemia hippocampus, karakterisasi fungsi memori spasial, volume hippocampus, jumlah neuron hippocampus, ekspresi RAGE dan BDNF hippocampus. Penelitian tahap 1 bertujuan untuk menentukan durasi iskemia (10, 15 dan 20 menit) dan durasi reperfusi (3, 7 dan 10 hari) yang paling optimal mempengaruhi volume iskemia hippocampus pada tikus normoglikemia. Penelitian tahap 2 untuk menentukan durasi hiperglikemia (1, 2, 3, dan 4 minggu) yang paling optimal mempengaruhi volume iskemia hippocampus pada tikus model iskemia global. Penelitian tahap 3 menentukan model diabetes iskemia berdasarkan parameter volume hippocampus, jumlah neuron CA1, CA2CA3 hippocampus, kadar BDNF dan RAGE hippocampus, dan fungsi memori spasial.

Metode. Rancangan penelitian adalah *post test control group design*. Subyek penelitian tikus jantan, sehat, berumur 3-4 bulan. Penelitian tahap 1, 2 dan 3 masing masing terdiri atas 10, 4 dan 4 kelompok. Model iskemia global diinduksi dengan teknik transient BCCAO. Model diabetes diinduksi dengan STZ 45 mg /kgBB dan NA 110 mg/kgBB. Volume iskemia hippocampus dihitung dengan metode Cavalieri pada sediaan otak dengan pewarnaan TTC. Volume dan jumlah neuron CA1 dan CA2CA3 hippocampus dihitung dengan menggunakan prinsip stereology estimasi jumlah. Kadar BDNF dan RAGE hippocampus dianalisis dengan ELISA. Fungsi memori spasial diamati dengan uji MWM.

Hasil. Volume iskemia hippocampus paling besar terdapat pada kelompok durasi iskemia 20 menit dengan reperfusi 7 hari (7,23±1,60 mm^3). Peningkatan durasi iskemia (koefisien korelasi 0,57, p 0,00) dan reperfusi (koefisien korelasi 0,30, p 0,03) akan meningkatkan volume iskemia hippocampus. Volume iskemia paling besar pada hippocampus tikus DMISKEMIA 4 (7,78±2,03 mm^3) dan DMISKEMIA 3 (6,74±1,79 mm^3). Peningkatan durasi hiperglikemia akan meningkatkan volume iskemia otak tikus model iskemia (koefisien korelasi 0,72, p 0,0004). Kadar RAGE lebih tinggi pada hippocampus tikus model diabetes iskemia (1637,57±70,99 pg/mL) dan diabetes daripada tikus kontrol normal (1450,42±104,78 pg/mL). Kadar BDNF lebih rendah pada hippocampus tikus model diabetes iskemia (298.46±95.07 pg/mL) dan diabetes (270,32±31,76 pg/mL) daripada tikus kontrol normal dan iskemia (545,62 ±196,70 pg/mL). Volume CA1 lebih kecil pada hippocampus tikus model diabetes iskemia (0,40 ± 0,11 mm^3) daripada tikus iskemia (0,59±0,11 mm^3) dan kontrol normal. Volume CA2CA3 lebih kecil pada hippocampus tikus model diabetes

iskemia daripada tikus kontrol normal. Jumlah neuron CA1 lebih rendah pada hippocampus tikus model diabetes iskemia (44.852±13.950) daripada tikus kontrol normal (116.934±15.860) dan iskemia (65.288±10.420; p=0,027). Jumlah neuron CA2CA3 lebih rendah pada hippocampus tikus model diabetes iskemia (65.400±8.500) daripada tikus kontrol normal (251.979±57.042). Memori spasial lebih rendah pada tikus diabetes iskemia, diabetes dan iskemia daripada tikus kontrol normal. TQT (15,40±6,66 detik; 10,80±3,96 detik; 15,40 ± 3,97 detik), panjang lintasan (27,66±8,63 cm; 19,18±7,84 cm, 22,04 ± 5,38 cm) dan frekuensi melintas (1,40±1,67 kali; 2,40±1,14 kali, 2,20 ± 1,48 kali) semua kelompok model diabetes iskemia, iskemia dan diabetes lebih kecil daripada kelompok normal. Kadar RAGE hippocampus dan GDP berkorelasi positif kuat satu sama lain dan berkorelasi negatif dengan kadar BDNF hippocampus, parameter histologi dan uji memori. Terdapat korelasi negatif kuat antara RAGE dengan semua parameter histologi. Kadar BDNF berkorelasi positif kuat dengan semua parameter histologi. Semua parameter histologi berkorelasi positif sangat kuat satu sama lain.

Kesimpulan. Model iskemia BCCAO dengan durasi iskemia 20 menit dan durasi reperfusi 7 hari adalah model yang paling optimal. Model Diabetes 4 minggu pada tikus iskemia adalah model yang paling optimal.

Kata Kunci. tBCCAO, Model Diabetes Iskemia, Volume Iskemia Hippocampus, Metode Hitung Titik, Metode Nv x Vref, Memori Spasial, Volume dan Jumlah neuron CA1, CA2CA3 Hippocampus, BDNF, RAGE.

ABSTRACT

Background. To improve correlation of animal-trial phase and clinical trial in drug development studies, standardized and characterization of animal model with comorbidity that mimics patient condition is needed. Differences in duration of artery clamping and duration of reperfusion in performing transient Bilateral Common Carotid Artery Occlussion (tBCCAO) techniques, a common technique used to induce brain ischemia in rats, results in large variation of reported ischemic volume. This study aims to characterize rat model of global brain ischemia in diabetic rat with variations in duration of ischemia, duration of reperfusion, duration of hyperglycemia. Point-counting method is used to unbiasely estimate hippocampal ischemic volume. Nv x Vref method is used to unbiasely estimate number of CA1 , CA2CA3 neurons hippocampus. Levels of BDNF hippocampus, RAGE hippocampus and spatial memory function examined.

Method. Adult male Wistar rats weighing 200-250 g were used in this study, 50 in the 1st phase and 20 in the 2nd-3rd phase. The global ischemia model was induced by tBCCAO with ischemic duration of 10,15 and 20 minutes and duration of reperfusion of 3, 7 and 10 days. In the 2nd phase, the selected variants of tBCCAO was performed in Streptozotocine-Nicotindamide-induced diabetic rats with fasting blood sugar (FBG) levels ≥ 126 mg/dL. The duration of hyperglycemia before ischemia induction surgery was 7, 14, 21 or 28 days. Measurement of hippocampus ischemic volume was performed using Cavalieri method on 2,3,5 Triphenyltetrazolium Chloride (TTC)-stained slices.The volume and number of neurons CA1 and CA2CA3 of the hippocampus are estimated using the stereology principle. Hippocampus BDNF and RAGE levels were analyzed with ELISA. Spatial memory function observed with MWM test. Statistical Analysis used One Way annova test and pearson correlation test.

Result. No significant difference in mortality rate between groups was observed. The 20 minutes ischemia – 7 day reperfusion group was selected for the 2nd phase because the ischemic hippocampal volume in this group (7.23 ± 1.60 mm^3) was larger than the volume in 20 minutes ischemia- 3 days reperfusion group (5.37± 1.70 mm^3; p= 0.1) while the ischemic hippocampal volume in both groups were significantly larger than the volume of ischemia in the other groups. In the 2nd phase, the largest ischemic hippocampus volume was found in the 28 days hyperglycemia group (7.78 ± 2.03 mm^3) which was not significantly different from the volume of ischemia hippocampus of 3 week hyperglycemia group (6.74 ± 1.79 mm3; p = 0.7). The ischemic hippocampus volume in both groups was larger than the volume found in the other groups with shorter hyperglycemia duration. Ischemic hippocampal volume was strongly correlated with duration of hyperglycemia (coefficient correlation 0.72; p=0.0004); duration of ischemia (coefficient correlation 0.57; p=0.00) and duration of reperfusion (coefficient correlation 0.30; p =0.03).RAGE levels were higher in the hippocampus of rat diabetic ischemia (1637.57±70.99 pg/mL) and diabetes than normal control rat (1450.42±104.78 pg/mL). BDNF levels were lower in the hippocampus of rat diabetic ischemia (298.46±95.07 pg/mL) and diabetes

(270.32±31.76 pg/mL) than normal control rat and ischemia (545.62 ±196.70 pg/mL). CA1 volume was smaller in the hippocampus of rat diabetic ischemia (0.40 ± 0.11 mm^3) than in ischemia rat (0.59±0.11 mm^3) and normal control. The volume of CA2CA3 was smaller in the hippocampus of rat diabetic ischemia than in normal control rat. The number of CA1 neurons was lower in the hippocampus of rat diabetic ischemia (44,852±13,950) than in normal control rat (116,934±15,860) and ischemia (65,288±10,420; p=0.027). The number of CA2CA3 neurons was lower in the hippocampus of rat diabetic ischemia (65,400±8,500) than in normal control rat (251,979±57,042). Spatial memory was lower in rat diabetic ischemia, diabetes and ischemia than normal control rat. TQT (15.40±6.66 seconds; 10.80±3.96 seconds; 15.40 ± 3.97 seconds), length pathway (27.66±8.63 cm; 19.18±7.84 cm, 22.04 ± 5.38 cm) and frequency of passing (1.40±1.67 times; 2.40±1.14 times, 2.20 ±1.48 times) all model groups of diabetic ischemia, ischemia and diabetes are smaller than the normal group. Hippocampus and GDP RAGE levels are strongly correlated with each other and are negatively correlated with hippocampus BDNF levels, histology parameters and memory tests. There is a strong negative correlation between RAGE and all histological parameters. BDNF levels are strongly correlated positively with all histological parameters. All histological parameters correlate very strongly with each other.

Conclusion. The 4-week hyperglycemia followed by 20 minutes clamping in tBCCAO surgery and 7 days duration of reperfusion is the most optimum model in inducing hippocampal ischemia.

Keywords.
tBCCAO, Diabetic-ischemia Models, Hippocampus ischemic volume, point counting method, Nv x Vref method, Spatial Memory, CA1 and CA2CA3 neurons estimated, BDNF, RAGE