Potensi neuroproteksi conditioned medium stem cell from human exfoliated deciduous (CM Shed) dalam upaya mencegah apoptosis progenitor neuron akibat induksi glutamat = Neuroprotection potential stemcell from human exfoliated deciduous conditioned medium (CM Shed) in preventing glutamate-induced neuron progenitor apoptosis.

Masagus Zainuri, author

Abstrak

ABSTRAK
Latar belakang. Eksitoksisitas merupakan salah satu mekanisme penting dalam kerusakan otak masa perinatal. Eksitoksisitas terjadi akibat peningkatan glutamat ekstrasel. Stem cell From Human Exfoliated Deciduous dapat mensekresi enzim GAD yang akan memgkatalisis perubahan glutamat menjadi GABA. Perubahan glutamat menjadi GABA menyebabkan kadar glutamat ekstrasel menurun, namun peningkatan GABA dapat menyebabkan terjadinya depolarisasi yang dapat berakibat timbulnya eksitoksisitas. penelitian bertujuan untuk menentukan potensi neuroproteksi CM SHED mencegah kerusakan progenitor neuron akibat induksi glutamat.Metode. Progenitor neuron diisolasi dari otak tikus umur 2 hari dan conditioned medium didapat dari MSC SHED. Sampel dibagi menjadi 4 kelompok, yaitu kelompok kultur progenitor neuron dengan medium neurobasal tanpa glutamat dan glisin N- , dengan medium neurobasal ditambah glutamat dan glisin N , dengan CM SHED tanpa glutamat dan glisin K- , dengan CM SHED ditambah glutamat dan glisin K . Pada progenitor neuron dilakukan pemeriksaan kadar mRNA GABAAR1, subunit NR2B NMDAR dengan RT PCR, kadar protein NMDAR1 dan GABAAR1 dengan ELISA. Caspase -3 dan 7AAD dengan Muse. Pada CM dilakukan pemeriksaan kadar GABA dengan Elisa.Hasil. Viabilitas progenitor neuron pada kelompok K 78,05 lebih tinggi dari kelompok kontrol N- 73,22 , sedangkan kelompok N lebih kecil 68,90 . Kelompok K memiliki kadar GABA paling tinggi dan berbeda bermakna dengan kelompok lainnya, sedangkan pada kelompok N paling kecil. Kadar GABA yang tinggi pada kelompok K diduga karena adanya enzim GAD yang dapat mengkatalisis perubahan glutamat menjadi GABA pada CM SHED. Hasil pemeriksaan mRNA GABAAR1 kelompok K paling tinggi dibandingkan kelompok lain, diduga disebabkan GABA dapat mengaktivasi jalur MAPK yang menyebabkan terjadinya proses transkripsi mRNA GABAAR1. Kadar protein GABAAR1 pada kelompok K paling kecil dibandingkan kelompok lain, hal ini diduga disebabkan karena adanya perubahan subunit dari 2 akibat peningkatan kadar GABA. Kadar GABA yang rendah pada kelompok N diduga disebabkan GABA banyak terpakai terikat dengan reseptor GABAAR1, sehingga terlihat rendah saat pemeriksaan. Hasil ini sesuai dengan pemeriksaan kadar GABAAR1, dimana pada kelompok N kadar GABAAR1 paling tinggi. Kemungkinan lain adalah adanya umpan balik negatif oleh GABAAR1. Pada pemeriksaan mRNA GABAAR1 kelompok N didapat penurunan ekspresi relatif terhadap kontrol. Diduga peningkatan protein GABAAR1 sudah tidak diperlukan sehingga ekspresi mRNA GABAAR1 dihambat. Pemeriksaan dilakukan setelah perlakuan selama 24 jam, diduga kondisi progenitor neuron telah mengalami fase akhir. Terdapat perubahan dinamik pada regulasi GABAAR1 progenitor neuron. Pada fase awal aktivitas eksitatorik merupakan hasil kerjasama GABAAR1 dan NMDAR, kemudian terjadi perubahan aktivitas GABAAR1 dari eksitatorik menjadi inhibitorik. Pada kelompok N diduga aktivitas inhibitorik GABAAR1 tidak dapat

mengatasi aktivitas eksitatorik akibat induksi glutamat, sehingga menyebabkan terjadinya apoptosis. Sedangkan pada kelompok K , dimana terdapat peningkatan GABA akibat CM SHED, aktivitas inhibitorik GABAAR1 dapat mengimbangi aktivitas eksitatorik oleh glutamat sehingga terjadi neuroproteksi.Kesimpulan. Dari hasil penelitian ini dapat disimpulkan CM SHED memiliki potensi neuroproteksi dalam mencegah apoptosis progenitor neuron akibat induksi glutamat.
<hr />
ABSTRACT
Backgound. Exitoxicity is one of the important mechanisms in perinatal period brain damage. Exitoxicity results from increased extracellular glutamate. Stemcell From Human Exfoliated Deciduous can secrete a GAD enzyme that will analyze the change of glutamate to GABA. The change of glutamate to GABA causes extracellular glutamate levels to decrease, but an increase in GABA can lead to depolarization which may result in the excitoxicity. the study aimed to determine the potential neuroprotection of CM SHED to prevent progenitor neuron damage of glutamate induction.Method. Progenitor neurons were isolated from the brains of mice aged 2 days and conditioned medium obtained from MSC SHED. The samples were divided into 4 groups, ie the progenitor culture group of neurons with neutral glutamate and neurobasal medium N- , with neurobasal medium plus glutamate and glycine N , with CM SHED without glutamate and glycine K- , with CM SHED plus glutamate and glycine K . In progenitor neuron, GABAAR1 mRNA, NR2B NMDAR subunit with PCR RT, protein NMDAR1 and GABAAR1 with ELISA were examined. Caspase -3 and 7AAD with Muse. In CM, GABA levels were evaluated with Elisa.Result. The viability of progenitor neurons in the K group 78.05 was higher than the control group N 73.22 , whereas the N group was smaller 68.90 . The K group had the highest levels of GABA and was significantly different from the other groups, whereas in the smallest N group. High GABA levels in the K group are thought as a result of the presence of GAD enzymes that can catalyze the change of glutamate to GABA in CM SHED. The highest level of GABAAR1 group compared to other groups was thought to be caused by GABA to activate MAPK pathway causing transcription of GABAAR1 mRNA. The level of GABAAR1 protein in the K group was the smallest compared to the other groups, presumably due to a subunit change from ? 2 due to elevated levels of GABA. Low levels of GABA in the N group are thought to be caused by GABA being widely used bound with GABAAR1 receptors, making it noticeably low during examination. This result is in accordance with the GABAAR1 concentration, which in the N group of GABAAR1 levels is highest. Another possibility is negative feedback by GABAAR1. On examination of group GABAAR1 mRNA N , there was a decrease in expression relative to control. It is suspected that the increase in GABAAR1 protein is not needed so that GABAAR1 mRNA expression is inhibited. The examination was performed after 24 hours treatment, presumably progenitor neuron has ending phase. There is a dynamic change in GABAAR1 progenitor neuron regulation. In the early phase of excitatory activity is the result of cooperation GABAAR1 and NMDAR, then there is a change of activity GABAAR1 from excitatory become inhibitory. In the N group it is suspected that GABAAR1 inhibitory activity can not overcome the excitatory activity by caused of glutamate induction, thus causing apoptosis. While in the K group, where there is an increase in GABA considering CM SHED, GABAAR1 inhibitory activity can compensate for the excitatory activity by glutamate resulting in neuroprotection.Conclution. From the results of this study can be concluded CM SHED has the potential of neuroprotection in preventing progenitor neuron apoptosis through glutamate induction.