

INTISARI

MORFOLOGI DAN PERSENTASE NEURON DOPAMINERGIK DARI KULTUR VENTRAL MESENSEFALON FETUS TIKUS PUTIH UMUR 14 HARI YANG DIPLATting DALAM MEDIA *MINIMUM ESSENTIAL MEDIUM* + 10% *HORSE SERUM* MENGGUNAKAN PEWARNAAN IMUNOSITOKIMIA DENGAN PENANDA TIROSIN HIDROKSILASE KONSENTRASI 1 : 1000

Tuti Tri Sedya Renaning Tyas
12/329592/KH/07341

Penyakit Parkinson merupakan penyakit neurodegeneratif karena rusaknya neuron dopaminergik di *substansia nigra pars compacta* (SNpc). Metode pengobatan yang tengah dikembangkan adalah *exogenous cell replacement*, namun terkendala karena jumlah donor jaringannya terbatas. Penelitian ini bertujuan mengetahui gambaran mikroskopik berupa persentase dan morfologi kultur neuron dopaminergik dari ventral mesensefalon fetus tikus putih umur 14 hari dengan pewarnaan imunositokimia pada media *minimum essential medium* (MEM) + 10% *horse serum* (HS) yang diharapkan mendapatkan metode kultur neuron dopaminergik yang optimal dan sederhana.

Ventral mesensefalon fetus tikus putih (*Rattus norvegicus albinus*) umur 14 hari dikoleksi pada tabung konikal berisi media MEM + 10% HS + 10% *fetal bovine serum*, kemudian didisosiasi secara mekanik dengan mikropipet, disentrifugasi selama lima menit pada kecepatan 200 g sebanyak dua kali, selanjutnya dipindahkan ke kultur primer (*flask*) dengan media yang sama sebanyak 2 ml lalu diinkubasi selama 60 menit dengan kadar CO₂ 5% pada suhu 37°C, kemudian ditambah 3 ml media dan diinkubasi kembali selama tiga hari. Kultur *diplating* pada plat berlapis kolagen 96 sumuran dengan media MEM + 10% HS selama tiga hari, dilanjutkan dengan pewarnaan imunositokimia metode *Labeled Streptavidin-biotin* (LSAB) dengan antibodi primer anti-Tirosin Hidroksilase *rabbit serum* (1:1000, Chemicon Int., Temecula, USA Cat No. AB152). Morfologi neuron dopaminergik diamati menggunakan mikroskop *inverted* perbesaran 10x20 dan dilakukan penghitungan persentase neuron dopaminergik terhadap seluruh sel yang tumbuh dengan perbesaran 10x10 pada 10 area acak tiap sumuran secara manual oleh tiga orang.

Persentase neuron dopaminergik yang tumbuh pada media adalah 10,4±14,2%/area. Morfologi neuron pada kultur yang teramati adalah neuron unipolar, neuron bipolar dan neuron multipolar.

Kata kunci: imunositokimia, kultur neuron dopaminergik, tirosin hidroksilase, ventral mesensefalon.

ABSTRACT

MORPHOLOGY AND PERCENTAGE OF DOPAMINERGIC NEURON FROM VENTRAL MESENCEPHALIC FETAL DAY 14 OF RAT CULTURE PLATTED IN MINIMUM ESSENTIAL MEDIUM + 10% HORSE SERUM MEDIA USING IMMUNOCYTOCHEMISTRY STAINING WITH 1:1000 TYROSINE HIDROXYLASE MARKER

Tuti Tri Sedyo Renaning Tyas
12/329592/KH/07341

Parkinson's disease is a neurodegenerative disease caused by progressive loss of the dopaminergic neuron in the substantia nigra pars compacta (SNpc). Exogenous cell replacement currently being developed as a treatment of Parkinson's disease, but the main problem remains the limited supply of donor tissue. The aim of this research is to find out morphology and percentage the dopaminergic neurons culture of fetal day 14 (Fd14) rat ventral mesencephalic using immunocytochemistry staining in minimum essential medium (MEM) + 10% horse serum (HS) media which is expected to optimize culture methods.

Ventral mesencephalon of Fd14 rat (*Rattus norvegicus albinus*) was collected and cultured in conical tube containing MEM + 10% HS + 10% fetal bovine serum media, then dissociated mechanically using micropipette. The tissue was centrifuged for five minutes at 200 g twice, then transferred to a primary culture (flask) containing the same media then incubated for 60 minutes at 37°C with 5% CO₂ level, afterwards 3 ml media was added to the culture and incubated for three days. Cultured cells then being plated on collagen-coated 96-wells tissue culture plate with MEM + 10% HS media for three days and then immunocytochemistry staining was done using Labeled Streptavidin-biotin (LSAB) method with primary anti-tyrosine hydroxylase antibody rabbit serum(1:1000, Chemicon Int., Temecula, USA Cat No. AB152). The neuron morphology was observed using inverted microscope with 10x20 magnification and the percentage number of dopaminergic neurons compared to entire grown cells was counted with 10x10 magnification on 10 areas/wells randomly by three persons.

The result showed that the percentage of dopaminergic neurons successfully grown in the media is 10,4±14,2%/area. The morphology of cultured neurons found were unipolar neuron, bipolar neuron and multipolar neuron.

Keywords: immunocytochemistry, culture of dopaminergic neuron, tyrosine hydroxylase, ventral mesencephalon.