Standard Operating Procedure

For

Gene transfer (nonviral and viral) into endogenous brain stem cells and grafting of ESC or IPSC into the brain.

PURPOSE

Potential new therapies for neurodegenerative diseases and brain injuries may involve (1) an activation of the existing endogenous brains stem cells (BSC) via gene transfer (Gene Therapy) and (2) grafting of the embryonic stem cells (ESC) or induced pluripotent cells (IPSC) into the brain. The purpose of these treatments is to restore neuronal population and the physiologic and behavioral functions of the brain. Parkinson's disease. We will collaborate with other investigators of the Western New York Stem Cell Culture and Analysis Center (WNYSTEM) who will provide mouse and human ESC and IPC for grafting into the brain. The proposed experiments are aimed to advance brain-regenerative therapies and help with their future application to the human conditions. The experiments will also test new methods of in vivo gene transfection (using nanoparticles), new neuroregeneration-promoting drugs as well as quantitative methods for the evaluation of brain function using micro PET, brain microdialysis, behavioral tests and stereological quantitative neuroanatomy.

2.0 SCOPE (Should include which Cores this SOP applies to)

This Procedure is designed to show you how to stereotaxically inject: nonviral-or viral DNA particles in the mouse brain

3.0 PROCEDURE

3.1 Stereotactic injection of nonviral or viral complexes.

Researchers will learn how to perform stereotactic injection of nonviral DAN –nanoparticle complexes (2-8 ul) complexed with DNA (0.08ug) into brain lateral ventricle. Purpose of this experiment will be to determine whether changes in dopamine neurons in 6-OHDA or in PQM model of the PD or PVD ischemia model may be prevented/reversed by transfection of potentially therapeutic plasmid DNAs. he mouse head is immobilized in stereotaxic frame by ear bars and nose cone. We use blunt-end ear bars which are not inserted inside the ear canal. Hence, the application of Marcaine into the ear canals is not necessary. Ocular lubricant is applied. The area around the site of incision is shaved. The scalp is scrubbed with soap ,then alcohol then betadine). An incision (approx.. ½ inch long) is made running along the dorsal surface of the mouse's head and the fascia are gently pushed aside to expose the skull. A small bur hole is drilled above the injection site using sterile bit. A 30 gauge stainless steel infusion needle connected to a Hamilton syringe is inserted into the brain lateral ventricle by lowering the stereotactic arm to precisely lower the needle into place. The unilateral intraventricular injection will be stereotaxically placed using micromanipulator screw at 1 ul/min. The wound is closed using standard sutures

for the skin and the Marcaine applied to the wound site (see 16F1). This surgery will take less than 20 min. During all stereotaxic surgeries performed in our lab, as well in this proposed study, we continuously monitor mouse's breathing and reflexes, therefore, we do not cover animal body with a drape

3.2 Stereotactic grafting of ESC or IPSC.

Researchers will learn how to perform stereotaxic grafting of human embryonic stem cells (ESC) and induced pluripotent cells (IPSC) or inject viral or nonviral DNA particles into the mouse or rat brain in vivo (in acute 6OHDA model or chronic Paraquat or LPS models of Parkinson Disease, or PVD model of ischemia). Grafting of the human ESC and IPC will be done into the brains of the immunodeficient male NSG mouse [NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/Szj] mice (25–35 g; The Jackson Laboratory). These mice (Behringer, Human-Animal; Chimeras in Biomedical Research, Cell Stem Cell 1, 2007; doi:10.1016 j.stem.2007.07.021) human xenografts intracranially at high rate. In addition, alternative protocols such as (i) use of NOD-1 nude mice or (ii) immunosuppression of normal c57Bl mice with cyclosporine A (10 mg/kg intraperitoneally) may be considered in the future. In such cases amendments to this protocol would be proposed.