Standard Operating Procedure
for
Acute Unilateral Mouse Model of Brain Ischemia Pia Vessel Disruption (PVD)

1.0 PURPOSE

This experimental model is used to test the therapeutic potential of endogenous and grafted stem cells, and gene- therapy on stroke/ischemia related diseases. Also, this model is used to determine the mechanism by which successful therapies ameliorate the symptoms of stroke/ischemia-related diseases including neurodegenerative diseases.

The basic design of all the experiments is:

1. Induce damage and assess deficits (behavioral, neurochemical, neuroanatomical)
2. Administer therapy (drug, stem cells, or gene therapy)
3. Assess recovery of function (behavioral, neurochemical, neuroanatomical)

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

This procedure applies to all personnel who will perform PVD surgery within the Stem Cells Engraftment and *in vivo* Analysis Core.

3.0 PROCEDURE

- Prep recovery area:
    - Obtain clean cages for animals with new bedding and environment (1 per animal)
    - Turn on heat lamp
    - Warm sterile saline

-Set-up stereotaxic and surgery area:
    -Spray and wipe down stereotaxic and table with Virox then ethanol
    -Check all tubing is properly connected
    -Turn on bead sterilizer *(we do not use this)*
    -Place diapers around stereotaxic unites

-Gather all items needed:
    -Sterile surgery tools
    -Q-tips, gauze
    -Scalpel, sutures
    -Ethanol, triadine solution
    -Tears and polysporin
    -Ketoprofen

-Anesthetize mouse with isoflurane at "5."
-Shave head, secure in ear bars (test that nose does not move left or right)
-Reduce isoflurane to "2."
- Rest animal torso on heating pad elevated so body and head is flat
-Wipe with ethanol, wipe with triadine, add tears to eyes
-Inject ketoprofen (0.1 ml/10mg; roughly 0.3ml) subcutaneously or IP
-Make incision anterior/posterior from between ears to base of skull
-Using Q-tips, gently rub skull to spread skin and remove any connective tissue
-Mark coordinates:
    -0.5ML, +2.5AP
    -3.0ML, +2.5AP

-3.0ML, -0.5AP
-0.5ML, -0.5AP
-Drill corners and then drill to connect, remove bone fragment
-Remove dura by gently grasping and pulling aside
-Soak Q-tips in saline and gently rub surface of brain
-Change Q-tip after each rub
-Continue until all vessels on surface have been removed (5-10 Q-tips)
-Allow bleeding to stop
-Should stop on own, if profuse, apply pressure with wet Q-tip
-Suture incision with polysporin
-Inject mouse with 1ml warm, sterile saline subcutaneously in two sites
-Place mouse in prepped cage under heating lamp
-Cover with wire, not plastic cover
-Allow to recover until walking around

4.0 DAMAGE

Mice with unilateral lesion of cortical pia vessels by PVD procedure will be tested for behavioral as well as for physiological and neuroanatomical abnormalities.

5.0 MICE EVALUATION

- Behavioral assessment of motor functions (see SOPs for behavioral tests)
- In vivo PET – measurement of blood flow and glucose metabolism (see SOP for PET)
- Immunocytochemistry – stereology assessment of neuronal loss and regeneration by endogenous or grafter stem cells (see SOP for stereology)

6.0 REFERENCES

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