Oct 18, 2019 Version 2

Induce Stroke via Hypoxia-Ischemia V.2

DOI
dx.doi.org/10.17504/protocols.io.8e5htg6

Ian Simpson[1]

[1]Pennsylvania State University

Diabetic Complications Consortium
Tech. support email: **rmcindoe@augusta.edu**


Lili Liang

DOI: dx.doi.org/10.17504/protocols.io.8e5htg6

External link: https://diacomp.org/shared/document.aspx?id=251&docType=Protocol

Protocol Citation: Ian Simpson 2019. Induce Stroke via Hypoxia-Ischemia. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8e5htg6

Protocol status: Working
We use this protocol and it's working

Created: October 18, 2019

Last Modified: October 18, 2019

Protocol Integer ID: 28861

Keywords: inducing stroke, Hypoxia-Ischemia, cardiovascular, neuropathy,

Abstract

Summary:

This document describes the procedure for inducing a stroke in diabetic RCS10 mice, their non-diabetic controls and NON/ShiLtJ control mice. Our initial objective was to use those RCS10 mice that did not become diabetic as the controls for the experiment to compare the effect of diabetes on stroke outcome and that data is illustrated in Figure 2. However, with further breading the numbers of non- diabetic RCS 10 mice at twenty weeks of age were so few that it became necessary to use the NON/ShiLtJ mice which are 85% genetically identical to the RCS10 mice and stroke outcome is illustrated in Figure 3.

Diabetic Complications:

Cardiovascular

Neuropathy

Materials

MATERIALS

MB10 **Quip Laboratories, Inc**

Bupivacaine **Mid West Vet Supply**

Bacdown **Fisher Scientific**

TTC **Merck MilliporeSigma (Sigma-Aldrich)**

Note:

Thermo Fisher Scientific (RRID:SCR_008452)

Sigma-Aldrich (RRID:SCR_008988)

1 Prior to H/I

All instrumentation and disposables utilized for the H/I procedure and/or recovery are autoclaved. MB10 disinfectant is used when instruments cannot be autoclaved.

2 Day of H/I

On the morning of experimentation, cages are wiped with MB10 and placed in a flow hood and the solid lid is replaced with an autoclaved filtered lid since it is several hours until the mice are returned to their home station, and the hood is too noisy to keep them in it for several hours. Animals are anesthetized with isoflurane (3% in 70% nitrous oxide/30% oxygen) in an induction chamber. The absence of a toe pinch reflex is used to determine a proper level of anesthesia. The mouse is then placed on the surgical bench and the nose is positioned in a nosecone to maintain a proper level of anesthesia using approximately 1.5% isoflurane in 70% nitrous oxide/30% oxygen. The neck is shaved and scrubbed with a 10% betadine solution, followed by a rinse with 70% ETOH, and the area is painted with 10% betadine. A local injection of Bupivacaine (5mg/kg) is made prior to making a small incision in the neck to expose the right carotid artery. Once exposed, the artery is double-ligated with 3-O surgical silk and the incision is sutured twice using Nylon monofilament suture. The animal is allowed to recover in his cage with access to food and water for 3 hours. Surgical instruments are placed in a Germinator 500 glass bead sterilizer for sterilization in between surgeries.

Following the 3h recovery from surgery, the mice are exposed to a predetermined hypoxic/ ischemic insult using 9% oxygen balance N2. Each mouse is placed in an individual 500ml glass jar within a closed system in a circulating water bath maintained at 35.5ºC. The water bath contains Bacdown as a disinfectant. Upon completion of the H/I insult, the mice are returned to their cages and monitored prior to being returned to the vent rack for the established period of recovery time. Mice are housed in pairs and are supplemented with 5% glucose in the drinking water. Food pellets are placed in the bottom of the cages for easy access, and a nestlet is provided for enrichment.

At the time of sacrifice, each mouse is placed in a restrainer. A 25GA needle is used to prick the tail, and 5ul of blood is collected into a glucometer cuvette to measure glucose levels. The mouse is then anesthetized with an IP injection cocktail consisting of 80-100mg/kg ketamine and 10mg/kg xylazine. A surgical plane of anesthesia is confirmed by the absence of a toe pinch reflex prior to immobilizing the mouse on a flat surface. An incision is made in the lower abdomen and continues up to the xyphoid process. At the

xyphoid, lateral incisions are made up towards the left arm and right arm near the neck. The rib cage is clamped back over the head, keeping it parallel with the torso. The descending aorta is snipped below the diaphragm to obtain serum or plasma, and the blood is collected into an appropriate tubes. A small incision is made in the left ventricle of the heart and a feeding tube is inserted up towards the ascending aorta so as the bulb sits directly beneath the junction of the aorta and heart. The aorta and feeding tube are clamped below the bulb of the feeding tube, and a small incision is made in the right atrium to begin perfusion. The mouse is perfused with 30ml of 1x PBS at a rate of 3.3cc/min.

Upon completion of the perfusion, the brain is removed from the skull and lowly immersed in isopentane maintained at 38ºC on dry ice. Once bubbling subsides, the brain is wrapped in a labeled piece of foil and stored on dry ice until the completion of the experiment and subsequently stored at -80ºC until cryosectioned. (Alternatively the fresh brain can be placed in a brain mold for TTC staining. See below.)16 µm Sections from fresh frozen or perfused flushed brains are placed on superfrost plus slides in a consecutive manner, such that two slides represent the entire areas of interest that includes the caudate putamen, cortex, striatum, hippocampus and thalamus. The slides are stored at -80ºC in a slide boxes until further analysis. 50 µm sections of the right hemisphere/ ipsilateral, and left hemisphere/contralateral are also obtained for mRNA and western blotting analysis.

3 Hematoxylin and Eosin Staining

Slides are taken from -80°C storage and placed in slide staining containers. Slides are hydrated in 2 min. changes each of 100%, 95%, 70% ETOH, then 2 min X2 dH2O, fixed in 70% ETOH for 5min, rinsed twice in dH2O for 2 min.. Sections are stained in freshly filtered Hematoxylin for 3 min. and rinsed in running tap water for 10 min. Slides are then dipped twice in Eosin and rinsed in several changes of 70% ETOH until ETOH stays clear. Slides are dehydrated in 95% ETOH 2×2 min., 100% ETOH 2×2 min., Xylene 3×2min., and then cover-slipped using a xylene based mounting media.

4 TTC Staining

The brain is removed from the skull and placed into a brain slicer mold placed in ice. Razor blades are positioned in the mold to obtain 2mm thick sections. Sections are placed in individual wells of a 24 well tissue culture dish with 500 µl of 2% TTC in 0.1M PBS. The plate is wrapped in foil and placed on a 37°C slide warmer for 30 minutes. TTC is removed and 500ul of fixative is added to each well for storage. Images of the sections are captured by Olympus S2H10 research stereo- microscope and Adobe

Photoshop software. The infarct area and total area of the contralateral and ipsilateral hemispheres are measured with Scion image software (Scion Corporation). The percent infarct area is calculated as a percentage of the ipsilateral hemisphere relative to the contralateral hemisphere as previously described (Swanson, Morton et al. 1990).

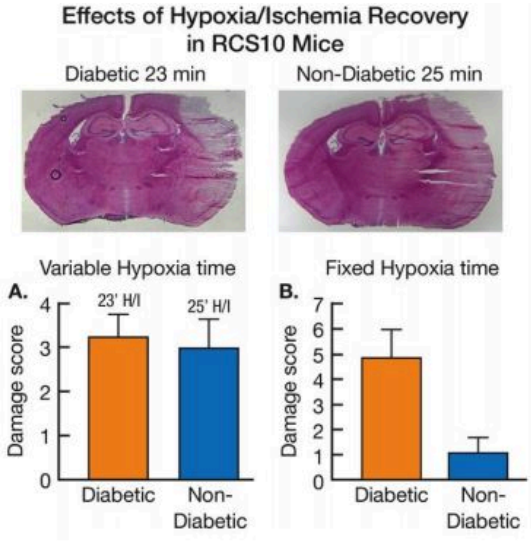
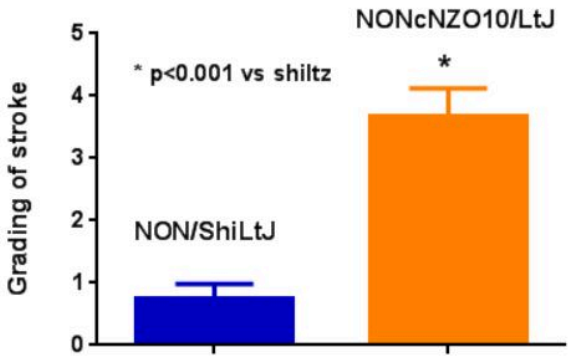


Figure 2: Upper panels illustrate typical H/E staining of diabetic and non-diabetic brains at 48 hours after H/I at a fixed hypoxia time of 23 min. (9% O2). Panel A indicates the quantification of the corresponding infarct volumes. Panel B illustrates a comparison between non-diabetic mice exposed 25 min. hypoxia (n=13) with diabetic min (n=20) exposed to 23 min. hypoxia. The infarct volumes are significantly smaller in the non-diabetic RCS10 mice and to normalize infarct volume to that of the RCS10 mice requires an additional two minutes of hypoxia.

5 Comparison of Infarct volume between diabetic NONcNZO10/LtJ (RCS10) and non-diabetic NON/ShiltJ mice



Comparison of Infarct volume between diabetic NONcNZO10/LtJ (RCS10) and non-diabetic NON/ShiltJ mice. This illustrates the infarct volumes that were determined by H&E in twenty week old RCS10 (n=23) and NONShiLtJ (n=28) mice that were exposed to 23 min of hypoxia /ischemia and allowed to recover for 48 h. The infarct volume is significantly smaller in the non- diabetic mice, which share 85% genetic identity with the diabetic RCS10 mice (Jackson Laboratories).