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Version 2

Neuropathy Phentoying Protocols - Insulin Implantation Protocol V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

Phenotyping of Rodents for the Presence of Diabetic Neuropathy

In man, the development of diabetic neuropathy is dependent on both the degree of glycemic control and the duration of diabetes. Diabetic neuropathy is a progressive disorder, with signs and symptoms that parallel the loss of nerve fibers over time. Consequently, assessments of neuropathy in mice are not performed at one time point, but are characterized at multiple time points during a 6 month period of diabetes. The degree of diabetes is evaluated in 2 ways: tail blood glucose measured following a 6 hour fast and glycated hemoglobin levels. The initial degree of neuropathy is screened using the methods discussed below. Detailed measures of neuropathy are employed when the initial screening instruments indicate a profound or unique phenotypic difference. This document contains protocols used by the DiaComp staff to examine and measure diabetic neuropathy at the whole animal, tissue and cellular levels.

Diabetic Complication:



Neuropathy



Materials

IMPLANT PRODUCT INFORMATION:

Trade name: LINPLANT (A sustained released insulin implant)

Composition: bovine insulin, palmitic acid.

Weight: About 26mg/ implant.

Dimension: 2 mm in diameter, 7mm long.

Release rate: ~2U/24hr/implant for >40days, subcutaneously.

Caution: Implant is not sterile. Implant should be immersed in diluted 2% povidine iodine or exposed to UV.
Do not put in alcohol.

Species: For rats use Linplant
For mice use Ultra-low dose linBit implant.

Troubleshooting



1 **Recommended**

Sites for Insertion: Subcutaneously in dorsal skin (back), neck-region.

2 Anesthesia:

Rat Intraperitoneal injection of .3ml undiluted ketamine- HCl (Ketastet) and xylazine mix (3parts of ketamine to 1 part of xylazine) for an average 350g rat.

Mice: Dilute .1ml of ketamine and xylazine mix (3 parts of ketamine to 1part of xylazine) to .9ml of .9% saline and inject .3ml i/p to an average 30g mouse.

Prep: The designated insertion site is shaved, cleansed and drenched with Betadine. The trocar and canula are immersed in 2 % Betadine solution. The skin is pierced with a 16G disposable needle while lifting up the skin between the thumb and index finger and withdraws the needle. The implant to be inserted is briefly immersed (about 10 sec) in 2% Betadine solution. The implant is then inserted into the proximal end of the trocar and the trocar pushed through the skin orifice/opening already created. The stylet is then used to push the implant until it exits from the distal end of the inserted trocar. The inserted implant is properly milked subcutaneously away from skin orifice. The insertion is soaked with Betadine solution. The skin defect should contract soon so no suture is required.

Dose: Two implants would render a diabetic rat weighing about 350 g non-diabetic. One (or one-half) implant would maintain a diabetic as diabetic.

A dose of sustained release insulin implant would last about 45 days.

Indication for use: For severely diabetic, poor-conditioned and cachexic rat whose blood glucose >450mg/dl or when the glucometer reading is high.

This is required especially when it is too early to sacrifice the rat for tissues.

Source: The Linplant comes in a research pack of 40 implants marketed by:

Linshin Canada Inc.

47 Marblemount Crescent North,

Ontario, Canada M1T 2H5.

Telephone: (416) 490-6634.

Fax: (416) 490-1535.

www.linshincanada.com

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