WVU IACUC Model Guidance Sheet:
Use of Streptozotocin (STZ) for Diabetes Induction in Animals

Purpose

STZ is a commonly used chemical agent for the induction of diabetes in research animals. This compound poses human health and safety risks and precautions ***must*** be taken. In addition, diabetes model is a chronic disease model which monitoring, supportive care and humane endpoints ***must*** be considered to ensure that appropriate animal welfare is maintained.

Definitions

Streptozotocin (STZ)- A compound derived from *Streptomyces achromogenes* with antineoplastic properties which target the insulin producing beta cells of the pancreas in mammals.

Carcinogen- A substance capable of causing cancer.

Teratogen- A substance which can cause malformation of an embryo/fetus.

Cytotoxic- A substance which is toxic to cells.

Guidance

1. Introduction
   a. STZ is a highly toxic compound. It is considered cytotoxic to pancreatic beta cells, suspected carcinogen, mutagen, teratogen, and reproductive toxin. There is no established safe occupational exposure limit.
   b. Potential routes of exposure include inhalation, ingestion, accidental injection, and dermal absorption.
   c. All safety procedures outlined by Environmental Health and Safety ***must*** be adhered to when working directly with this compound or handling animals which received this compound. Please follow the IACUC SOP "*Hazardous Chemicals Used with Animals*"
   d. Animals are considered to be hazardous for 72 hours after last administration of STZ. After the 72-hour period, animals can be transferred back into standard housing rooms.
2. Reagents
   a. STZ is commonly obtained as a non-pharmaceutical grade compound (*see IACUC Policy "Non-Pharmaceutical-Grade Substances Used in Animals"*) from vendors such as Sigma-Aldrich®.
   b. STZ is typically mixed in a citrate buffer solution to obtain a final compound with a pH of 4.5 - 5.5.
   c. Compounds ***must*** be prepared using sterile technique.
   d. All reagents used and a description of the compounding process ***must*** be described in the animal use protocol.
   e. Compounding considerations:
      i. STZ should be stored at -20° C.

ii. STZ container should be protected from light due to potential degradation.
iii. STZ is unstable and should be mixed with citrate buffer immediately prior to injection. It ***must*** be prepared fresh each time and given within 5 minutes of mixing.

3. Common Dosing Regimens
   a. STZ can be used to induce either Type I or Type II diabetes depending on the administration regimen used.
   b. Sensitivity to compound can vary with animal age, strain/stock, and sex used. A pilot study may be necessary to determine appropriate treatment regimen. Below are some commonly used protocols to provide a starting point, but regimens may need to be adjusted based on response.
   c. Some diabetes induction protocols require a short fasting period prior to STZ administration.
      i. Standard fasting is 4 hours for mice and 6-8 hours for rats.
   d. Rats
      i. Type I diabetes model
         – Single dose 40-70 mg/kg (65 mg/kg commonly used dose) in rats 8-10 weeks of age
      ii. Type II diabetes model (STZ + Nicotinamide)
         – Nicotinamide (230 mg/kg IP) followed by STZ (65 mg/kg IV)
         – Nicotinamide protects against complete beta cell toxicity
      iii. Type II diabetes (fat fed STZ model)
         – High fat diet (60% fat by caloric content) for 3 weeks prior to STZ administration
         – STZ given on day 22 (40 mg/kg IP)
   e. Mice
      i. Repeated low dose Type I diabetes model
         – 40 mg/kg IP once daily for 5 consecutive days
         – This model has fewer toxic effects
      ii. Single high dose Type I diabetes model
         – Single IP injection 200 mg/kg

4. Prior to administration of STZ
   a. Ensure animals are transferred into a chemical hazard room and Office of Laboratory Animal Resources (OLAR) staff has appropriate documentation (Animal Hazard Form and MSDS). Animals receiving hazardous chemicals ***must*** be housed in a designated chemical hazard room and cannot remain in the standard housing. Contact OLAR husbandry managers for guidance on this process.
   b. STZ injections and all animal manipulations (during first 72 hours post-STZ administration) ***must*** be performed under a certified class 2A or 2B biosafety cabinet or chemical fume hood.
   c. Animals ***must*** be properly restrained for injection. Chemical restraint reduces risk of potential exposure of personnel to hazardous agents.
   d. All animals should be weighed on day of experiment to ensure accurate dosing of STZ compound.

5. STZ administration
   a. STZ should be administered as described in the approved IACUC animal use protocol.
   b. After injection, animals should be returned to their cage with adequate food and 10% sucrose water (prevents post-injection hypoglycemia). Sucrose water should be maintained on cage for 48-72 hours post-injection.

6. Post-STZ administration monitoring and considerations
    a. Animal use protocol should outline frequency and method used for blood glucose (BG) testing.
        i. Fasting is typically performed prior to BG testing. The NIH and Animal Models of Diabetic Complications Consortium (AMDCC) recommend a morning fast starting at 7 am with an afternoon blood collection. Fasting recommendation is 6 hours for mice and 6-8 hours for rats.
    b. Animal use protocol should outline expected BG concentration once animal is diabetic and any compounds administered to reduce BG.
    c. Monitor for clinical signs of hypoglycemia during first 48 hours.
        i. Lethargic
        ii. Unresponsive
        iii. Contact veterinary staff to assist with treatment
    d. Recommend daily monitoring for the first week post injection, and weekly monitoring once stable.
    e. Diabetic animals have an increased water intake need, please ensure adequate water supply is available.
    f. Diabetic animals may require an increased cage change frequency due to increased urination.
    g. Clinical signs of diabetic animals:
        i. Polydipsia (increase water intake)
        ii. Polyuria (increase urination)
        iii. Abdominal distention
        iv. Dehydration
        v. Weight loss and body condition loss
        vi. Unkempt appearance
    h. Diabetic animals can benefit from supportive care including nutritional support (soft chow/diet gel), food on ground/dish on cage floor, fluid support, heat support, group housing, nesting material/huts. A special care form should be submitted to OLAR staff describing additional care which is needed.
    i. Routine monitoring (weighing, body condition score (BCS), hydration checks, BG measurements) and humane early endpoints ***must*** be described in the animal use protocol.

References

https://currentprotocols.onlinelibrary.wiley.com/doi/epdf/10.1002/cpz1.78