Guidelines on Use of Streptozotocin in Rodents

Last Updated 8 March 2013

1. Purpose
To describe the appropriate use of streptozotocin for induction of diabetes mellitus (DM) in rats and mice and associated husbandry procedures
To describe the appropriate post-injection monitoring of rodents

2. Responsibility
1. Investigators using STZ to induce diabetes in rodents

3. Definitions
1. Streptozotocin (STZ): An antitumor/antibiotic compound isolated from *Streptomyces achromogenes* with potent toxicity to beta islet cells of the pancreas
2. Carcinogen: An agent capable of causing cancer
3. Teratogen: An agent capable of causing developmental abnormalities
4. Cytotoxic: Possessing ability to exert toxic effects on a cell

4. Background
1. **STZ Uses and Properties**
   1. Depending on the dose and dose frequency, STZ can be used to induce insulin-dependent Type 1 DM or non insulin-dependent Type 2 DM.
   2. Known to cause hepatic and renal toxicity in mice and rats. Due to toxic effects and disease progression, some animal mortality can occur. However, steps to reduce mortality must be taken.
   3. Is a carcinogen, teratogen, and has been shown to affect fertility in animal studies.
   4. Effect on different strains is variable. ULAM recommends starting at the lower end of the dose ranges until performance for a given strain can be established.
2. **Common Sequelae in DM Models**
   1. Increased appetite, defecation, thirst, and urination
      1. Appropriate availability of water must be ensured (e.g., if water bottles are being supplied, consider providing two bottles).
      2. Cage change frequency may need to be increased to account for increased urination.
   2. Kidney injury
   3. Cardiovascular disease
   4. Genitourinary infection
   5. Vision loss
3. **Rat Dosages**
   1. Recommended doses vary considerably due to strain, age, and weight differences in susceptibility to STZ, as well as variation in the bioactivity of the STZ itself. A general range for use is 42-65 mg/kg administered intraperitoneally (IP). The compound may also be administered intravenously (IV) or by other less common routes.
4. **Mice Dosages**
   1. As with rats, strain susceptibility varies considerably and vendor variation may be significant when using outbred stocks. Even variation between vendors has been documented. The dose range of STZ for mice is much higher than rats: 100-200 mg/kg IP.

5. Procedures
1. **STZ Preparation and Administration**
1. Reagents and Solutions

As long as non-pharmaceutical grade reagents are approved in the UCUCA protocol, use reagent-grade materials obtainable from various commercial vendors such as Sigma-Aldrich.

1. Streptozotocin
2. Sodium citrate
3. Citric acid
4. 10 mM sodium citrate and 10 mM citric acid are used to create the citrate buffer as the solvent for the STZ. Sodium citrate and citric acid are combined until a pH of 4.5-5.5 is obtained.
5. Sucrose is combined with standard drinking water to make a 10% solution to be placed in water bottles and provided to each cage.

2. Protocol

1. Procure animals to allow for adequate acclimation time.
2. The night before the injection, fast all animals to maximize the effectiveness of the STZ. Drinking water must still be provided.
3. Attain accurate body weights to administer an exact dose. Younger, lighter animals are dosed at the higher end of the dose range than the older, heavier animals (due to higher metabolic rate).
4. Prepare STZ using sterile technique immediately prior to injection following proper procedures as outlined in Animals Administered a Hazardous Substance Requiring Containment to reduce STZ exposure to personnel.
   1. STZ is weighed and dissolved in citrate buffer (based on amount needed calculated for doses to be administered).
   2. Note: Recent studies suggest that acidifying the STZ is an unnecessary step in preparing the solution in order to induce diabetes.
5. Animals are injected using a 0.5 inch 25 gauge needle on a tuberculin (1.0 ml) syringe.
   1. Ensure accurate placement of the needle by aspirating (pulling back on the syringe plunger) before injecting:
      1. For IP, IM, SQ injection, the injection should not be administered if blood or discolored fluid is drawn back into the syringe.
      2. For IV injection, the injection should not be administered unless blood is drawn back into the syringe confirming IV access.
   3. If the injection is not administered, discard the needle, syringe, and syringe contents, draw up fresh STZ using a new needle and syringe and attempt another injection.
5. A new needle and syringe should be used for each animal.

3. Post-injection Procedures and Monitoring

1. Provision of sucrose water during the induction period can reduce morbidity and mortality. Upon completion of the injections, animals are given 10% sucrose water to drink. This is important because the initial cytotoxic destruction of the beta islet cells causes an excessive release of insulin into the bloodstream. If not provided the 10% sucrose water, the animals can experience a potentially fatal hypoglycemia. The 10% sucrose water is typically provided for 48 hours.
2. If the animals become hypoglycemic in spite of the presence of sucrose water, they should be given a 1.0 ml bolus of the sucrose water by gavage. Clinical signs of hypoglycemia include lethargy and slowed responsiveness, progressing to recumbence and coma. Severe cases of hypoglycemia may require veterinary intervention and IV glucose administration. Hypoglycemia is expected 8-24 hours after STZ injection.
3. Test animal blood glucose 48 hours post STZ injection; if they are hyperglycemic at this time, they can be returned to standard drinking water.
4. Animals should be monitored daily during the induction period and at least weekly, once the animal has stabilized.
   1. Monitoring is imperative so that deterioration in health can be noted and addressed in a timely manner.
   2. The End-Stage Illness Scoring System should be used to determine appropriate endpoints.

6. Related Documents

1. Animals Administered a Hazardous Substance Requiring Containment
2. End-Stage Illness Scoring System

7. References