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Induction of Type 1 Diabetes, Myocardial Infarction, Echocardiography, Measurement of Blood Glucose and Tissue Weights V.2



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We use this protocol and it's working

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Abstract

Summary:

This protocol presented an induction of Type 1 Diabetes, and myocardial Infarction, performed an evaluation of *in vivo* residual LV function by echocardiography, and measurement of blood glucose and tissue weights of rats.

Diabetic Complication:



Cardiovascular

Troubleshooting

1 **Induction of Type 1 DM**

Type 1 DM was induced in male Sprague–Dawley rats by administering a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (65 mg/kg body wt) (Sigma) prepared daily in citrate buffer pH 4.5 for maximal stability. The control group was injected with the vehicle only. Development of DM was confirmed 48 hours later by the presence of glycosuria (>2000 mg/dl) along with polyuria. Rats with urine glucose values of <2000 mg/dL 24–48 hours after STZ injection were not considered to be diabetic and were excluded from further study.

2 **Induction of Myocardial Infarction**

Diabetic and non-diabetic male Sprague–Dawley rats (200–225 g body weight) were anaesthetized intraperitoneally with Nembutal (40 mg/kg). Absence of a response to pinching the toe was used as an indicator of the appropriate level of anesthesia. Rats were then rapidly intubated and mechanically ventilated (tidal volume, 1 ml/100 g body weight; ventilation rate, 65 strokes/min) by a constant volume small animal ventilator (Model 683, Harvard Apparatus). A left thoracotomy was performed at the fourth intercostal space, and the left anterior descending (LAD) coronary artery was then ligated by irreversible tightening of a 6–0 suture loop. Myocardial infarction (MI) was confirmed by regional cyanosis of the myocardial surface distal to the suture, accompanied by S-T segment elevation on the electrocardiogram. Following successful induction of MI, the chest cavity was compressed to evacuate any air before being tightly sealed. The rats were given buprenorphine (0.05 mg/kg) pre-operatively, then Q 8–12 hours post-operatively by subcutaneous injection for 48 h.

3 ***In vivo* Residual LV Function Evaluation by Echocardiography**

Transthoracic echocardiographic images of hearts from all groups of rats were obtained using an ultra high-resolution ultrasound scanner (Vevo 2100; VisualSonics, Inc.) under light anesthesia. For M-mode recordings, the parasternal short-axis view was used to image the heart in two dimensions at the level of the papillary muscles to obtain LV fractional shortening (LVFS) and LV ejection fraction (LVEF). All measurements were averaged in five consecutive cardiac cycles and analyzed off-line by a single blinded observer using software resident on the ultrasonograph. All calculations were derived using standard formulas. LV internal dimension during diastole and systole (LVIDd and LVIDs) were measured from M-mode tracings obtained at the mid-papillary level and analyzed according to modified American Society for Echocardiography standards.

4 **Measurement of Blood Glucose**

Blood samples were collected from the tail vein for the determination of blood glucose levels using Accutrend Plus test strips and meter (Roche).



5 **Tissue Weights**

Pieces of tissues from the lungs and liver were removed and weighed. For the determination of dry weight, these were placed in an oven at 65°C until a constant weight was reached. Ratios of wet to dry weight were calculated for both lungs and liver.