Restoration of euglycemia in the RCS10 mice with Metformin

Edited by: Ian Simpson

(note that the following list should be linked to the appropriate location.)

Summary: In this series of studies we wished to determine whether Metformin was able to establish and maintain euglycemia in RCS10 mice and would reduce infarct volume following a stroke. Metformin is a widely prescribed agent to treat Type II diabetes and as illustrated in Figure 2, when Metformin (0.8-1.3 g/kg/D) is included in the water, it is able to establish and maintain euglycemia in the RCS10 mice over a 4-week period. However, as illustrated in in Figure 3, restoration of euglycemia with metformin did not improve ischemic outcome.

Reagents and Materials:

(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)

Reagent/Material	Location	Quantity Required	Vendor	Stock Number
Metformin			Sigma was the preferred vendor	
saccharin			Fisher	

Protocol:

WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.

The Metformin was administered to the mice in their water to which 0.15% saccharin was added to ensure that mice consumed sufficient metformin/water to normalize their

blood sugar (0.8-1.3 g/kg/D). The saccharin levels in the water of control mice were diluted to normalize saccharin consumption. Figure 3 describes the effects of the Metformin on stroke outcome, which was determined at 48 h after the infarct by H/E staining as described in the earlier report. These results in some respects are disappointing as our expectations were that the metformin would elicit a comparable effect to that we had obtained when euglycemia was induced in the *ob/ob* mouse with darglitazone, a *ppar* γ agonist. In that study, darglitazone not only normalized the blood glucose levels it dramatically reduced the infarct volume in both diabetic and non-diabetic animals. The data described in Figure 3 is comparable to that obtained by (Tureyen et al., 2007) who found that Metformin treatment in the *db/db* mouse had no effect on stoke outcome. However, in those studies the *db/db* mice were too insulin-resistant to fully restore euglycemia and thus the observations were always considered equivocal. It should also be noted that the levels of Metformin used in this study and that of Tueyen et al. is significantly higher on a mg/kg basis than used in patients, suggesting marked difference in sensitivity between rodents and humans which was not the case with the *ppar* γ agonists. This represents a very important observation that will lead will alter the scope of the RO1 resubmission as we will now not propose to investigate the mechanistic aspects of metformin but we have clearly demonstrated unlike the *db/db* mice the glycemic state in RCS10 can be modulated.

Figure 2

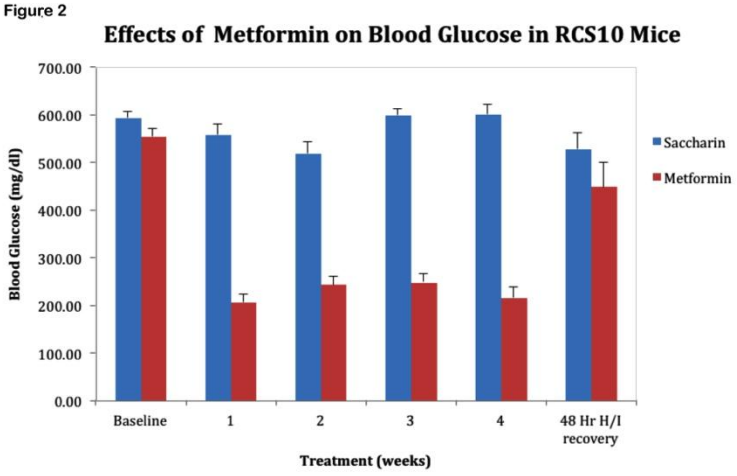

Figure 2 describes the time course for the normalization of blood glucose in the RCS10 mice. The RCS10 mice were 20 weeks old when Metformin treatment was initiated and had been diabetic for at least 4 weeks To maintain euglycemia the Metformin concentration in the drinking water was increased from 100 -120 mg/ml over the course of the 4 weeks. 0.15% saccharin was added to mask Metformin and the concentrations were adjusted to ensure equal saccharin intake in the control RCS10 mice. The Metformin was unable to maintain euglycemia following the stroke presumably due to elevated corticosterone and consequent increased insulin resistance.

Figure 3

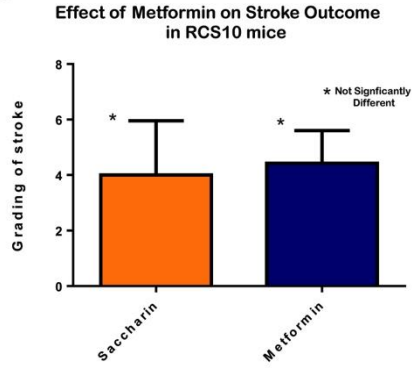

Figure 3 illustrates the effects of maintaining RCS10 mice euglycemic for four weeks on the subsequent outcome following stroke in the mice maintained as described in Figure 2. Both sets of animals were exposed to 22 min of 9% oxygen. The extent of the stroke damage was determined at 48 h post stroke infarct from H&E sections as illustrated in earlier report. (n=8, Saccharin control mice and n=15, Metformin mice). There was no significant difference in the extent of the insult between the diabetic and euglycemic mice.

1. Perform step one.
Explanation of what happens in this step.

IMPORTANT: Important comments precede steps that, if performed incorrectly, will result in damage to equipment or to the samples.

2. Perform step two.

   a. Perform sub-step (a)

   Amplifying or more detailed information about the step.

   b. Perform sub-step (b)

NOTE: Additional tip that improves efficiency.

3. Perform step three.

Reagent Preparation: *(This area may have several different preparations with the table of contents below.)*

Reagent 1:

Reagents and Materials
Procedure
Checklist

Reagent 2:

Reagents and Materials
Procedure
Checklist

Reagent 3:

Reagents and Materials
Procedure
Checklist