EVALUATION OF THE ZUCKER FATTY RAT AS A MODEL OF DIABETIC NEUROPATHY

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Introduction

Diabetic neuropathy (DBN) is the most common clinical form of neuropathic pain and is associated with numbness, tingling sensation and pain (Callahan et al., 2012). One of the most common approaches to model DBN in rodents is the streptozotocin (STZ) model. STZ is an antibiotic that is diabetogenic due to a direct cytotoxic effect on pancreatic beta cells. STZ treated rats rapidly develops signs of diabetes such as elevated blood glucose levels and a reduced ability to tolerate glucose, but not necessarily a thermal stimulus. However STZ rats also develop marked signs of general ill health which raises concerns about the validity of the model (Fox et al., 1999).

In the search for alternative models of DBN, we have examined the Zucker fatty rat, a genetic model of type 2 diabetes. Although the Zucker rat is widely used as a model of obesity and type 2 diabetes, relatively few reports have described this model in the context of pain (Bruseeu et al., 2008; Otto et al., 2011; Lupachyk et al., 2012; Vera et al., 2012). These studies represent an investigation of two cohorts of Zucker rats (Charles River, strain code 185, Crl: ZUC-Lep-r fa) and their lean controls (strain code 186) in which we specifically assess their value as a model of diabetic neuropathy. We also compare to the Spered Nerve Injury (SNI) model of neuropathic pain (Decosteed and Woolf, 2000).

General characterisation of the Zucker rat

Male, Zucker rats (Charles River; strain code 185, Crl:ZUC-Lep-r fa) and their lean controls (strain code 186) were made to characterise the Zucker rat relative to its lean control.

Body composition: QMR and MRI imaging

Zucker lean Zucker fatty

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Zucker Lean</th>
<th>Zucker Fatty</th>
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<tbody>
<tr>
<td>3 months</td>
<td>180.0±22.6</td>
<td>162.0±13.4</td>
</tr>
<tr>
<td>6 months</td>
<td>210.0±14.8</td>
<td>194.0±16.7</td>
</tr>
<tr>
<td>12 months</td>
<td>250.0±18.2</td>
<td>235.0±18.0</td>
</tr>
<tr>
<td>18 months</td>
<td>300.0±15.3</td>
<td>280.0±15.0</td>
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MRI images taken of a Zucker rat compared to its lean control (age 7 month) show an increase in both subcutaneous fat (green) and particularly visceral fat (red), when compared to its lean control.

At 10 months of age, the Zucker rat demonstrated a significant tactile allodynia relative to Zucker lean controls (P<0.01: T-test). The magnitude of the allodynia was confirmed by repeated measures ANOVA with a 3-way factorial: Zucker, Diabetes, and Treatment. All Zucker rats developed evidence of type 2 diabetes as indicated by an elevated OGGT response, hyperglycemia and hyperlipidemia compared to lean controls. However not all rats were necessarily neuropathic as approximately 15% Zucker rats did not develop DBN.

Cohort 1

A group of 20 Zucker rats (20 males aged 10 months) and 9 age-matched, Zucker lean (2) counterparts were study subjects. The Zucker rats were confirmed diabetic as measured by elevated blood glucose, increased water intake and heightened responses to an oral glucose load.

The rats were then evaluated in a battery of tests to measure their responses to a variety of nociceptive stimuli. Specifically:

A: Thermal hyperalgesia
B: Mechanical allodynia
C: Acetone drop test
D: Insulin sensitivity

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Cohort 2

A group of 16 Zucker rats aged 3 months, and 12 age matched, Zucker lean (ZL) counterparts were study subjects. The Zucker rats showed considerably lower spontaneous locomotor activity compared to lean controls. This difference was evident from earliest testing (3 months). Also similar to cohort 1, within animal comparisons failed to identify any correlation between blood glucose level and locomotor activity. Zucker rats developed an age dependent tactile allodynia that appeared to only reach a robust magnitude compared to lean controls after 9-12 months of age. This development of tactile allodynia was independent of various biomarkers indicative of diabetes (blood glucose, OGGT, blood insulin) which was examined in cohorts 1 and 2. In contrast, Zucker rats showed elevated paw withdrawal thresholds to the von Frey test in both cohorts 1 and 2. This indicated that tactile allodynia was occurring in the absence of changes in nociceptive thresholds, a feature that is consistent with earlier studies (Lupachyk et al., 2012).

Pharmacological characterisation: Zucker vs. SNI model of neuropathic pain

Male Zucker rats from cohorts 1 and 2 (approximate age 10-12 months) were evaluated for their response to pregabalin, lacosamide and carbamazepine. In parallel, these same drugs were tested in a group of age matched Sprague-Dawley rats with Spared Nerve Injury (SNI) model (Decosteed and Woolf, 2000) briefly, make SD rats 4x sciatic nerve injury prepared by sectioning the tibial and peroneal branches of the sciatic nerves, leaving the sural branch intact. Sham animals underwent similar surgical procedures except the sciatic nerve was not sectioned and a decompressing branches of the nerve remained intact. Following a recovery period of at least 20 days, the animals were familiarised to the test procedures which included measurement of tactile allodynia (von Frey).

All experiments were conducted according to a repeated measures design with 2-4 days between each treatment cycle. Blood was collected from the ear veins to determine plasma glucose. Zucker rats with Van Frey scores >10 were excluded from any drug study.

Summary

1. Two cohorts of Zucker rats were profiled and each found to develop an age related tactile allodynia that was sensitive to pregabalin.

2. The diabetic condition clearly preceded the tactile allodynia which only reliably emerged at 9-12 months age. Similar to the SNI rat model, no thermal allodynia was evident.

3. The Zucker rat may represent a useful alternative to surgical models of neuropathic pain, such as the SNI model. A comparison between each model is summarised below.

References:


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