Pro-inflammatory TNF and HMGB-1 Increase During Diabetic Neuropathy: The Effect of Decreasing Levels of TNF in the Brain on HMGB-1 Production

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Abstract

Neuropathic pain (NP) is a form of chronic pain that results in damage to the nervous system. Diabetic neuropathy is a debilitating complication of diabetes mellitus and is the most frequent form of neuropathic pain encountered in the developed world. Damaged neurons release an inflammatory protein termed high-mobility group box chromosomal protein 1 (HMGB-1). HMGB-1 is a nuclear protein; however, when released from cells it has pro-inflammatory cytokine-like activity. An increase in the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF) underlies nerve dysfunction and death, and development of NP. HMGB-1 released from damaged neurons stimulates production of TNF. While diabetes is associated with elevated HMGB-1 levels, whether this contributes to diabetic neuropathy is unknown. TNF-silencing nanoplexes are effective for relieving diabetic NP. These nanoplexes knock-down TNF levels in the brain and decreased pain. We hypothesize that the TNF nanoplexes will also indirectly reduce HMGB-1 levels, contributing to the reduction in diabetic NP.

Background

- Tumor necrosis factor-alpha (TNF), when administered i.p. to rats, increases the perception of pain [1].
- Streptozotocin (STZ)-injections induce diabetic neuropathy [2].
- TNF levels were increased in the hippocampus during diabetic neuropathy [3].
- Increased levels of TNF in the brain and enhanced TNF production by macrophages are associated with pain development [2].
- HMGB-1 levels in serum increase during diabetic neuropathy [4].
- Enzyme-linked immunosorbent assay (ELISA) detects HMGB-1 concentrations [4].
- Gold nanoparticles (GNPs) complexed with small inhibitory ribonucleic acid (siRNA) is termed nanoplexes [5].
- Nanoplexes protect siRNA from degradation [6].

Previous Methods

Male Sprague-Dawley rats, 175-250 gms, are administered a single intraperitoneal injection (45 mg/kg) of streptozotocin (STZ).

Behavioral testing for pain (mechanical allodynia) for 3 days prior to (baseline) and once per week after STZ injection for 60 days.

Rat weights are monitored every other day; blood glucose readings are performed prior to, on day 4 post-STZ, and once a week thereafter for the duration of the study.

Upon sacrifice: blood is collected, and brain regions are harvested, processed, and stored until assayed for TNF; levels of TNF are assessed using WEHI bioassy (bioactive protein).

Peritoneal macrophages are harvested by lavage and plated in multi-well Lab-Tek for short-term in vitro experiments; assess the effect of lipopolysaccharide (LPS; 30 ng/ml) induced TNF production.

Supporting Data

**Macroimmune TNF Production**

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF Production (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No LPS</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>STZ-14 days</td>
<td>250 ± 20</td>
</tr>
<tr>
<td>STZ-21 days</td>
<td>300 ± 20</td>
</tr>
</tbody>
</table>

**Mechanical Allodynia**

Average of 3 Measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>Mechanical Allodynia (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>STZ-14 day</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>STZ-21 day</td>
<td>20 ± 1</td>
</tr>
</tbody>
</table>

**Effect of Nanoplexes on Blood Glucose Levels**

TNF: tumor necrosis factor-alpha; HMGB-1: high-mobility group box chromosomal protein 1; GPRS: gold nanoparticle.

**Cellular Mechanism**

Glucose → AGE formation → HMGB-1 → Neuronal Damage

**Future Outlook**

- Continue optimizing standard curves for macrophage and serum samples.
- Continue running macrophage and serum samples to increase n numbers.
- Run collected nanoplex samples to compare to TNF data.
- Create a standard curve for homogenized tissue, and run samples to compare to current HMGB-1 data.

**Results**

**Macromolecular HMGB-1 Production**

<table>
<thead>
<tr>
<th>Group</th>
<th>HMGB-1 Production (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>400 ± 50</td>
</tr>
<tr>
<td>Saline</td>
<td>500 ± 50</td>
</tr>
<tr>
<td>STZ</td>
<td>700 ± 50</td>
</tr>
</tbody>
</table>

**Conclusions**

- Peritoneal macrophage stimulated with LPS increases HMGB-1 production (Fig. 6).
- Peritoneal macrophages from STZ-DN rats are more pro-inflammatory than those from STZ-NR and saline control rats (Fig. 7).
- STZ-DN rats show increased HMGB-1 production when stimulated with LPS (Fig. 7). *Due to low n numbers, statistical analysis not performed.

**Acknowledgements**

- Research supported by Center for Undergraduate Research and Creative Activities.
- Special thanks to the members of the Ignatowski lab and Davidson lab.

**References**