Effect of triiodo-L-thyronine on axonal regeneration in the rat spinal cord after acute compression injury

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Studies were performed on the effect of triiodo-L-thyronine (T3) on clinical recovery and axonal counts in the pyramidal tract of 56 rats subjected to an acute spinal cord compression injury at T-7. The T3 was given at a daily dose of 5 μg/kg for 4 weeks to 28 rats in the treatment group. The treatment and control animals were tested weekly for clinical recovery, and cord function as determined by the inclined-plane technique. Groups of animals were killed at 4 weeks and 12 weeks, and the axons in the pyramidal tract cephalad and caudad to the injury site were counted in sections prepared with Holmes' silver stain.

There was no difference in clinical recovery between the treatment and control groups. This negative result contrasts with other studies which showed improved recovery of cord-injured animals treated with thyroid hormones. The possible explanations for this discrepancy are discussed. Similarly, there was no difference in the axon counts between the treated and control groups. Thus, T3 did not improve recovery or axonal regeneration in the pyramidal tract of rats after acute spinal cord compression injury. Between 4 and 12 weeks, there was a marked reduction in the cephalad axon counts in the pyramidal tract in both groups, indicating that approximately 50% of the axons in the pyramidal tract had undergone retrograde degeneration or dying back by 12 weeks after this degree of injury. The T3 did not affect the degree of retrograde degeneration.

KEY WORDS • spinal cord injury • thyroid hormone • axonal regeneration • pyramidal tract

Axonal regeneration can be defined as regrowth of the proximal axonal stump after the axon is severed, with establishment of functional synapses. Such regeneration takes place in the spinal cord of lower animals, and in the peripheral nerves of higher animals, including man. Although some axonal regeneration takes place in the spinal cord of higher animals, whether restoration of somatic motor or sensory function takes place remains controversial. There are several possible reasons for the difference between higher and lower animals in their capacity for central axonal regeneration. An inhibiting factor may be present centrally in higher animals: this originally was postulated to be a physical factor such as scar tissue, but has recently been considered to be a biochemical factor, possibly produced by glial cells. Other possibilities include an inability of some or most central neurons to reestablish functional synapses. In this regard, it should be noted that transplants of certain embryonic central neurons in vertebrates have grown and established functional synapses in the brain and spinal cord. Thus, there is evidence that central axons can regenerate and redevelop functional synapses under specific conditions, even in vertebrates.

One substance thought to be involved in axonal growth and regeneration is thyroid hormone. The presence of thyroid hormone is necessary for the normal initial development of the central nervous system in the fetus and newborn. In addition, thyroid hormone has been reported to influence the regeneration of damaged axons in the brain and peripheral nerves. The effects of thyroid hormone on regeneration have been reviewed recently by Kiernan. In 1967, Harvey and Srebnik reported that L-thyroxine improved the recovery of rats after crush injuries of the spinal cord, and in our laboratory it was recently shown that thyroid hormones produced
a slightly improved recovery in rats subjected to an acute cord compression injury. 35

The present experiment was undertaken to further assess the clinical and histological effects of triiodo-L-thyronine (T3) in spinal cord-injured rats. Clinical assessment was made by the inclined-plane technique, and histological assessment was performed by counting axons in the pyramidal tract proximal and distal to the site of injury, a technique recently developed in our laboratory.

Materials and Methods

Experimental Protocol

Fifty-six female albino rats of the Wistar strain* underwent an acute compression injury of the spinal cord at the T-7 level with a modified aneurysm clip.† The clip compressed the cord for 1 minute at a compression force of 174 gm, a method of injury developed in our laboratory.7,28 Immediately after injury, each animal was randomly assigned to a treatment or control group consisting of 28 animals each. In the treatment group, the rats received a daily intraperitoneal injection of T3,‡ for 4 weeks at a dose of 5 µg/kg body weight given as a 1-µg/ml solution in normal saline. The injection was prepared by dissolving T3 powder in NaOH and NaCl to yield a solution of 30 µg/ml, as described by Green.10 The daily dose was similar to that used in our previous study,35 but in that study treatment was administered for only 2 weeks. The control animals received an equivalent volume of normal saline (5 ml/kg/day for 4 weeks, administered intraperitoneally). All animals required manual bladder expression, and any urinary tract infections were treated with intramuscular gentamicin. Other aspects of care, including maintenance of ambient temperature at 28°C, were similar to our previous study.35

Clinical and Histological Assessment. The rats were assessed weekly by the inclined-plane technique, which records the highest angle from the horizontal at which an animal can maintain its position on an inclined plane for 5 seconds.26 Normal animals score better than 80° on the plane. Tests were made blindly without knowledge of the experimental group of a given animal. Ten animals from each group were sacrificed 4 weeks after injury, and their spinal cords were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 6 µm, and prepared with Holmes' silver stain for histological assessment by the axon-counting method, and with Luxol-fast blue.

The remaining animals were sacrificed 12 weeks after injury, and the spinal cords prepared similarly.

Axon counting was performed "blindly" without knowledge of the experimental group of a given animal. Counts were made on cross sections of the spinal cord taken from sites 6 mm cephalad and 6 mm caudad to the injury site. Counts from more distant sections were taken if distortion of the tissue or extensive infarction interfered with the ability to count axons at the 6-mm sites. The counts were made through an eyepiece grid and ×100 oil-immersion lens. In each section, all the axons were counted in at least two areas of the pyramidal tract, each measuring 625 sq µm. The pyramidal tract was chosen for axon counting, since in the rat this tract is distinctive both in appearance and location. It contains a high proportion of unmyelinated fibers, packed very closely together, and is located in the ventral aspect of the dorsal columns.1,18,25,33 It was previously found that the normal pyramidal tract in the thoracic region contains approximately 250 axons in a 625-sq µm section (unpublished data). The difference in axon count between the treatment and control groups was assessed by Student's t-test.

Results

Clinical Assessment

Table 1 shows the mean scores on the inclined plane for the surviving animals in the treatment and control groups for each week after injury. There were no significant differences in recovery between the two groups (p > 0.05) as determined by the performance of the animals on the inclined plane.

### Table 1

Clinical assessment of recovery of surviving spinal cord-injured animals in T3-treated and control groups

<table>
<thead>
<tr>
<th>Weeks After Injury</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Rats</td>
<td>Recovery</td>
<td>No. of Rats</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>47 ± 10</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>46 ± 8</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>49 ± 7</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>49 ± 9</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>51 ± 9</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>49 ± 8</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>46 ± 11</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>48 ± 11</td>
</tr>
</tbody>
</table>

* Female albino rats of the Wistar strain were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.
† Aneurysm clip manufactured by Walsh Manufacturing Ltd., Oakville, Ontario, Canada.
‡ T3 obtained from Sigma Chemical Co., St. Louis, Missouri.
TABLE 2
Axonal concentration at pyramidal tract sites 4 and 12 weeks after injury in T3-treated and control groups

<table>
<thead>
<tr>
<th>Weeks After Injury</th>
<th>Relation to Injury Site</th>
<th>Treatment Group</th>
<th>Control Group</th>
<th>Axonal Counts*</th>
<th>Axonal Counts*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Rats</td>
<td>No. of Rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>cephalad</td>
<td>10</td>
<td>10</td>
<td>292 ± 58</td>
<td>256 ± 68</td>
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<tr>
<td></td>
<td>caudad</td>
<td>10</td>
<td>10</td>
<td>72 ± 28</td>
<td>73 ± 18</td>
</tr>
<tr>
<td></td>
<td>cephalad minus</td>
<td>10</td>
<td>10</td>
<td>220 ± 45</td>
<td>183 ± 67</td>
</tr>
<tr>
<td>12</td>
<td>cephalad</td>
<td>9</td>
<td>8</td>
<td>126 ± 39</td>
<td>135 ± 55</td>
</tr>
<tr>
<td></td>
<td>caudad</td>
<td>9</td>
<td>8</td>
<td>50 ± 14</td>
<td>58 ± 20</td>
</tr>
<tr>
<td></td>
<td>cephalad minus</td>
<td>9</td>
<td>8</td>
<td>76 ± 43</td>
<td>77 ± 58</td>
</tr>
</tbody>
</table>

* Axonal counts are expressed as the number of axons in 625-sq \( \mu m \) areas of the pyramidal tract. Values are means ± standard deviation.

Histological Assessment by Axon Counting. As is shown in Table 2 and Fig. 1, at both 4 and 12 weeks after injury there was a major reduction in the axon counts in the pyramidal tract caudad to the injury site in both the treatment and control groups. For example, at 4 weeks, the cephalad count was 292 axons/625-sq \( \mu m \) section and the caudad count was 72. However, at neither 4 nor 12 weeks was there any significant difference between the treatment and control groups in axon counts cephalad to the lesion, or caudad to the lesion, nor in the difference between the two sites (p > 0.05). For example, at 12 weeks, the treatment group had a cephalad-caudad count difference of 76 ± 43 axons, and the control group had a difference of 77 ± 58 axons. In both the treatment and control groups, the cephalad counts at 12 weeks were significantly lower than the cephalad counts at 4 weeks, although there were no significant differences between the caudad counts at the two study times (Table 2 and Fig. 1).

Pathological Changes. The cord sections showed several major pathological changes. One was the expected Wallerian degeneration of axons in the pyramidal tract caudad to the injury site and in the ascending tracts in the dorsal columns and elsewhere cephalad to the injury site. These degenerated tracts were shrunken and showed a marked decrease in the number of darkly staining axons and contained some swollen, lightly stained parts of axons. Another frequent finding was the presence of discrete infarcts, often extending longitudinally for several segments, especially in the center of the dorsal columns. At 4 weeks, both treatment and control groups showed a major reduction in the cephalad counts.

**Fig. 1.** Axon counts in the pyramidal tract of control and T3-treated animals at 4 and 12 weeks after injury. For each group the counts for individual animals at sites cephalad and caudad to the injury site are shown. The vertical line adjacent to the results for each group shows the group mean and standard deviation. The lack of significant difference between the treatment and control groups is evident. At 12 weeks, both treatment and control groups showed a major reduction in the cephalad counts.
weeks the injury site usually showed large areas of necrosis, whereas at 12 weeks the injury site showed glial proliferation and collagenous scarring, often related to the adjacent dura. At both times after injury, syringomyelic cavities were seen, especially centrally, in the gray matter and adjacent white matter. Occasionally, infarcts or cavities involved or encroached upon the pyramidal tracts and interfered with the counting of axons. The pathological changes were similar in the treatment and control groups.

**Discussion**

Clinical assessment by the inclined-plane technique showed no significant difference in functional recovery between the treatment and control groups. Our previous study with T3 was performed under somewhat different conditions and showed a slight, but significant, increase in recovery of the treated animals at 12 and 16 weeks after injury. If this improved recovery after administration of T3 had been due to regeneration of pyramidal tract axons, including the formation of functional synapses, these regenerated axons should have been histologically apparent at 12 weeks or earlier in the present study. The axon counts caudad to the injury site should have been higher in the T3-treated animals. The fact that the present experiments showed no differences in the caudad axon counts between the treatment and control groups suggests that the difference in clinical scores in the previous study was not due to pyramidal tract regeneration. In the previous experiment, the injury was performed at T-1, whereas in the present study the animals were injured at T-7. It is possible that the T-1 injury caused some weakness of the upper limbs in addition to weakness of the legs, because these animals achieved much lower scores on the inclined plane than the animals injured at T-7. For example, at 12 weeks the control animals injured at T-1 achieved 32° on the inclined plane, whereas the control animals in the present study achieved 48° (Table 1). In the former study, thyroid hormone may have facilitated local segmental recovery such as reinnervation of the forelimbs rather than long-tract regeneration. However, this mechanism would not account for the original observation of Harvey and Srebnik, who found improved recovery of leg function in rats with injuries at T-10 treated with thyroid hormone.

The axon-counting technique also showed that there were no significant differences between the treatment and control groups in the concentration of axons in the pyramidal tract at either 4 or 12 weeks. Thus, the number of axons distal to the lesion did not increase as a result of treatment with T3. Possible explanations for this failure include a lack of effect of the hormone on axonal regeneration in the pyramidal tract, insufficient dosage of T3 (either total amount or duration), or failure of penetration of the hormone into the injury site. It has been shown that acute cord compression causes severe posttraumatic ischemia and infarction of the injured cord segment and the adjacent cephalad and caudal segments.

In both our former study, in which significant improvement in clinical recovery was evident at 12 and 16 weeks after injury (p < 0.05), and the study of cord compression by Harvey and Srebnik, in which improvement was also demonstrated, treatment with T3 was stopped 2 weeks after injury; however, in the present study it was continued for 4 weeks. It is unlikely that the longer period of treatment had a negative effect on the present results, because it is known that, in hyperthyroid states, regenerative changes in peripheral nerves are accelerated.

An unexpected finding was the marked decrease in axon counts cephalad to the injury site which occurred between 4 and 12 weeks after trauma. One possible explanation is that the cephalad axon stumps which were beginning to regenerate at 4 weeks subsequently degenerated by 12 weeks. It is of interest that, although Sala and Ramon y Cajal described this dying-back phenomenon many years ago, quantitative data on its extent in the spinal cord have not been reported. Kalil and Schneider transected the pyramidal tract in the medulla of hamsters and showed that retrograde axonal degeneration progressed in a cephalad direction for several months, reaching as far as the cerebral peduncle 10 to 14 months after injury.

Another explanation would be to attribute the decrease in cephalad axons to continuing and progressive cord destruction secondary to the original injury. There is some evidence for this, such as the frequent presence of infarction, cavitation, and scarring which often appeared to extend from a degenerated tract to encroach on an adjacent non-degenerated tract. Spreading edema, venous thrombosis, or pressure from an adjacent syringomyelic cavity may also be involved.

**Conclusions**

The present experiment showed no beneficial effect from intraperitoneally administered T3 on the functional or histological recovery of rats after spinal cord injury. The role of vascular injury and scarring secondary to degeneration may be significant in furthering the effects of a compression injury and preventing the process of axonal regeneration. Axonal degeneration appears to evolve for several weeks following an acute compression injury of the cord.

**Acknowledgments**

The technical assistance of Mrs. L. Marmash and Miss Bev Michelson is gratefully acknowledged.

**References**


Manuscript received August 9, 1982.

Funds for this research were provided by the Medical Research Council of Canada (MT-4046).

Dr. Rivlin was a Fellow of the Medical Research Council of Canada.

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