Effect of Thyrotropin-Releasing Hormone on the Time Course of Neurologic Recovery after Spinal Cord Injury in the Rat

Tadatoshi HASHIMOTO and Naohisa FUKUDA
Research and Development Division, Takeda Chemical Industries, Ltd.,
2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532, Japan

Accepted May 2, 1990

Abstract—Spinal cord injuries in rats were experimentally produced by compressing the cord at the eleventh thoracic vertebral level for 60 minutes with stainless steel screws (2 mm in diameter and 2.8 mm in length). The main neurologic signs produced by cord compression were motor as well as sensory deficits and urinary incontinence. Rats with a neurologic score, based on both motor and sensory deficits, of 1 (complete paraplegia but responsive to tail pinching) 24 hours after injury were used to study the relative effect of subcutaneous treatment with TRH once or twice daily for 7 consecutive days on the time course of recovery after spinal cord injury and the dose-dependency of this effect. Once daily (5, 15, or 45 mg/kg/day) or twice daily (2.5, 7.5, or 22.5 mg/kg×2/day) treatment with TRH starting 24 hours after injury improved the neurological signs and reduced the incidence of urinary incontinence, dose-dependently. The minimum effective doses for once and twice daily treatments were 45 mg/kg/day and 7.5 mg/kg×2/day. These results indicate that the neurologic recovery-accelerating effect of TRH administered 24 hours after cord injury for 7 days is dose-dependent and that a twice daily dosage schedule tends to produce better improvement in the neurologic state than a once daily schedule dose.

Thyrotropin-releasing hormone (TRH) was originally identified as the substance that causes the release of thyrotropin and prolactin from the pituitary gland (1). TRH has many other actions on the central nervous system (2, 3) and has been used in the treatment of several neurological and psychiatric disorders (4–10). Faden and collaborators (11) have shown that a 4-hour intravenous infusion of TRH starting 1 hour or even 24 hours after traumatic spinal cord injury improves the extent of the neurologic recovery in cats. Aii and collaborators (12) have shown that TRH administration improves clinical parameters in patients with spinal cord injury, both chronic and stable cases. TRH has therefore been receiving a great deal of attention because of its possible use as pharmacotherapy for spinal cord injury. Although the neurologic outcome-improving effect of TRH administered as late as 24 hours after spinal cord injury is noteworthy (13), TRH treatment starting 24 hours after injury has not been studied in detail except in cats (11).

The aim of the current work is to reconfirm the neurologic outcome-improving effect of TRH treatment starting 24 hours after spinal cord injury using another species, model (14) and administration method to study the dose-dependency of TRH’s effect, and to compare the relative effect of two TRH dosing schedules.

Materials and Methods

Animals: Male Wistar rats weighing about 300 g were used. They were kept under constant environmental conditions (24±1°C and a regular 12-hour light/dark cycle), given food and water ad libitum, and were housed in groups of 4.

Surgical procedures and postoperative care: A midline longitudinal incision was made into the skin of the back under sodium pentobarbitone (30 mg/kg, i.p.) anesthesia,
the fascia was cut along the midline, and the
dorsal surface of the spinal column was ex-
posed from T10 to T12 by paramedian in-
cision into the paraspinal muscles. The
spinous process at the eleventh thoracic (T11)
vertebra was dissected, and a small burr hole
was made at the center of the dissected sur-
f ace of the vertebra. A stainless steel screw
(2 mm in diameter and 2.8 mm in length) was
implanted into the burr hole made just above
the dura mater, and then the incision was
sutured. On the following day, rats showing
no neurologic dysfunction were anesthetized
by ether inhalation, and the sutures were cut
to expose the head of the screw. The spinal
cord was compressed by inserting the screw
as far as possible. The screw was left in place
for 60 minutes. Following the removal of the
screw, the incision was resutured. Rats having
urinary incontinence were housed individual-
ly, and any urine wetting the external skin was
washed away daily with warm water, and the
area was dried with a paper towel.

Neurologic symptoms and scoring: The
main neurologic symptoms caused by com-
pression-induced spinal cord injury were
motor deficits, sensory deficits, and urinary
incontinence. Neurologic scores (NS) of 0–5
based on both motor and sensory deficits,
were defined as follows: 0: no spontaneous
movement of the hindlimbs and no withdrawal
response upon tail pinching, including avoid-
ance movements by forelimbs, biting of
clamps or vocalization; 1: no spontaneous
movement of the hindlimbs but showing a
withdrawal response upon tail pinching, in-
cluding avoidance movement of the forelimbs,
biting of clamps or vocalization; 2: barely
perceptible coordinated movement of the
hindlimbs and forelimbs; 3: well coordinated
movement of the hindlimbs and forelimbs, but
no weight bearing by four limbs; 4: able to
walk with weight bearing by all four limbs, but
with an ataxic gait; 5: normal walking. Urinary
incontinence was judged by wetness around
the external genitalia due to urine. An inves-
tigator who was unaware of each individual
animal’s treatment evaluated the neurologic
functions 24 hours and 2, 3, 4, 5, 6, 7, 8, 10,
and 14 days after removal of the screws. Days
required for the increase in the NS to a value
greater than the initial NS of 1 were also
recorded.

Administration of TRH: The rats with an
NS of 1 twenty-four hours after cord injury
started receiving subcutaneous injections of
TRH (thyrotropin-releasing hormone tartrate,
Takeda) or saline. The injections were given
once (9:00 AM) or twice (9:00 AM and 3:00
PM) daily for 7 consecutive days. Saline or
TRH dissolved in saline was injected in a
volume of 1 ml/kg just after neurologic
scoring.

Analysis of data: NSs of the animals in the
saline-treated control group were compared
with those of the animals in the TRH-treated
groups utilizing the non-parametric Mann-
Whitney U-test (two-tailed). Differences in
the incidence of urinary incontinence were
compared utilizing Fisher’s exact probability
tests (two-tailed). Other differences were
compared utilizing Dunnett’s multiple range
test (two-tailed) following analysis of vari-
ance (ANOVA).

Results

The main results of the spinal cord injury
produced in rats by compression at the level
of the T11 vertebra using stainless steel
screws were motor deficits of the hindlimbs,
sensory deficits of the hindlimbs and tail, and
urinary incontinence. Eighty-two percent of
the animals with an NS of 1 twenty-four
hours after injury showed urinary incon-
tinence. All the animals used survived more
than 14 days after the spinal cord injury.

Effect of once daily administration of TRH
on the neurologic deficits in rats with spinal
cord injury: As shown in Table 1, the mean
number of post-injury days required for the
increase in the NS in the rats with spinal cord
injury that were treated with saline and TRH
5, 15, and 45 mg/kg once daily for 7 con-
ssecutive days starting 24 hours after injury
was 5.7, 4.0, 3.9, and 3.0, respectively, in
indicating that TRH had a significant dose-
related accelerating effect on the increase in
the NS to a value greater than the initial score
of 1 (F(3,52)=3.70, P<0.05). The acceler-
ation of the recovery rate in the TRH, 45 mg/
kg-treated group was significant when com-
pared to the saline-treated control (T=3.26,
P<0.01). The median and individual NSs for
the group treated with saline and those
Table 1. Number of days required for TRH administered once or twice daily to increase the neurologic score (NS) in rats with spinal cord injury to a score higher than the initial score of 1.

<table>
<thead>
<tr>
<th>Frequency of administration of TRH</th>
<th>Saline</th>
<th>TRH (mg/kg/day, s.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once daily</td>
<td>5.7±1.0</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td>Twice daily</td>
<td>4.4±0.4</td>
<td>3.5±0.3</td>
</tr>
</tbody>
</table>

The administration of TRH was started 24 hours after the spinal cord injury. The observation period was 14 days. Each value represents a mean±S.E. Number of animals in each group was 14. *P<0.05, **P<0.01, compared to the respective saline-treated control (Dunnett’s multiple range test, two-tailed).

treated with TRH at 5, 15, and 45 mg/kg-treated group at 3, 5, 7, and 14 days post-injury are shown in Fig. 1. TRH had an accelerating effect on the neurologic recovery after spinal cord injury. There was a significant difference between the saline-treated control group and the TRH, 45 mg/kg-treated group at each post-injury observation from day 3 to day 14 post-injury. As shown in Fig. 2A, a recovery-accelerating effect of once daily treatment with TRH was also observed with respect to the urinary incontinence, and the reduction in the incidence of urinary incontinence was significant in the TRH, 45 mg/kg-treated group when compared to the saline-treated control group from day 4 to day
14 post-injury.

Effect of twice daily administration of TRH on the neurologic deficits in rats with spinal cord injury: As shown in Table 1, the mean number of post-injury days required for the increase in the NS in spinal cord-injured rats that were treated with saline and TRH at 2.5, 7.5, and 22.5 mg/kg twice daily for 7 consecutive days starting 24 hours after injury was 4.4, 3.5, 3.2, and 3.0, respectively, indicating that TRH had a significant dose-dependent accelerating effect on the increase in the NS to a value greater than the initial score of 1 (F(3,52)=3.86, P<0.05). The recovery accelerating effect in the TRH, 7.5 mg/kg x 2 (T=2.76, P<0.05) and 22.5 mg/kg x 2 (T=3.09, P<0.01)-treated groups was significant when compared to the saline-treated group. The median and individual NSs for the saline and TRH, 2.5, 7.5, and 22.5 mg/kg x 2-treated groups at 3, 5, 7, and 14 days post-injury are shown in Fig. 3. There were significant differences between the saline-treated control group and the saline-treated control group and the TRH, 22.5 mg/kg x 2-treated group from day 5 to day 14 post-injury. As shown in Fig. 2B, a dose-related recovery-accelerating effect of twice daily treatment with TRH was observed with respect to urinary incontinence, and the reductions in the incidence in the TRH, 7.5, and 22.5 mg/kg x 2-treated groups were significant when compared to the saline group from day 4 to day 14 and from day 3 to day 14 post-injury, respectively.

Comparison of the relative effect of once and twice daily repeated treatment with TRH on the neurologic recovery: Total NS, obtained by the summation of the NSs from 9 observations (from day 2 to day 14 post-injury), in the groups which were treated once and twice daily are shown in Fig. 4. The median total NS in both saline-treated control groups was 19, indicating that the severity of the motor and sensory deficits in the two control groups was almost the same. The median total NSs in the groups that were treated once and twice daily with 15 mg/kg/day of TRH were 23 and 24.5, respectively; and there was
a significant difference between the control group and the group treated twice daily with 15 mg/kg/day of TRH. On the other hand, the median total NSs in the groups that were treated once and twice daily with 45 mg/kg/day of TRH were almost the same. In addition, while the mean number of days required for 45 mg/kg/day of TRH administered once and twice daily to elevate the NS above the initial NS of 1 was 3.0 in both cases, these numbers for 15 mg/kg/day of TRH administered once and twice daily were 3.9 and 3.2, respectively (Table 1). This was also true for the reducing effect of TRH on the incidence of urinary incontinence (Fig. 2).

**Discussion**

The present study demonstrated the accelerating effect of TRH on the sensory-motor recovery after thoracic spinal cord compression-induced injury in rats when it is administered subcutaneously once or twice daily for 7 consecutive days starting 24 hours after injury. This accelerating effect was dose-dependent, and the minimum effective dose for once and twice daily treatment was 45 mg/kg/day and 7.5 mg/kg×2/day, respectively. Moreover, this is the first study to demonstrate a reducing effect of TRH on the incidence of urinary incontinence, one of the main neurologic signs seen after spinal cord injury in the present model.

The median total NS for the animals that were treated twice daily with 15 mg/kg/day of TRH, which is the intermediate daily dose in the present study, was higher than that for the animals that were treated once daily with the same daily dose of TRH. On the other hand, those in the groups that were treated once and twice daily with 45 mg/kg/day of
TRH, which is the highest dose, were almost the same. In addition, the mean days required for 45 mg/kg/day of TRH administered once and twice daily to elevate the NS above the initial NS of 1 was 3.0 in both cases, while that for 15 mg/kg/day of TRH administered twice daily was smaller than that in the group that was treated once daily with the same daily dose of TRH. Thus, it is suggested that a TRH administration method that exposes the animal to TRH more frequently may be preferable when treatment with TRH begins 24 hours after spinal cord injury and the dose of TRH is intermediate. In addition, our preliminary study showed that continuous administration of TRH, 15 mg/kg/day, for a week using an osmotic mini-pump caused a significant acceleration of the neurologic recovery in rats with an NS of 1 twenty-four hours after spinal cord injury (T. Hashimoto et al., unpublished).

TRH, which is the highest dose, were almost the same. In addition, the mean days required for 45 mg/kg/day of TRH administered once and twice daily to elevate the NS above the initial NS of 1 was 3.0 in both cases, while that for 15 mg/kg/day of TRH administered twice daily was smaller than that in the group that was treated once daily with the same daily dose of TRH. Thus, it is suggested that a TRH administration method that exposes the animal to TRH more frequently may be preferable when treatment with TRH begins 24 hours after spinal cord injury and the dose of TRH is intermediate. In addition, our preliminary study showed that continuous administration of TRH, 15 mg/kg/day, for a week using an osmotic mini-pump caused a significant acceleration of the neurologic recovery in rats with an NS of 1 twenty-four hours after spinal cord injury (T. Hashimoto et al., unpublished).

Faden and collaborators (11) first showed that intravenous infusion of TRH (2 mg/kg bolus, followed by 2 mg/kg/hour for 4 hours, totally 10 mg/kg) 24 hours after cervical spinal cord injury that is induced by weight-drop improves the extent of the motor recovery in cats. The present study reconfirmed the neurologic outcome-improving effect of TRH treatment starting 24 hours after spinal cord injury. However, Faden et al. have demonstrated this effect of TRH only with a 4-hour infusion, while our administration method was once or twice daily injection of TRH. In pilot experiments, we failed to demonstrate a significant neurologic outcome-improving effect with a single subcutaneous dose of TRH (45 mg/kg) 24 hours after injury using the present model, but with once daily subcutaneous treatment with TRH (45 mg/kg/day) for 7 days starting 24 hours after injury, we have succeeded in demonstrating a significant effect. Thus, in the present study, we adopted repeated treatment with TRH for 7 days.

A number of pathophysiological factors have been proposed as the cause of the post-traumatic necrosis seen in spinal cord injury following the initial insult (13, 15). It has been
reported that TRH antagonizes the behavioral changes induced by β-endorphin (16, 17), one of the proposed pathophysiological factors (18), and reverses the hypotension induced by an arachidonic acid metabolite, leukotriene D₄ (19), also one of the pathophysiological factors (20). Accordingly, it is conceivable that the neurologic recovery-accelerating effect of TRH shown in the present study is a result of the antagonistic action of TRH on the changes induced by the pathophysiological factors mentioned above. However, it has been assumed that the irreversible disruption of the spinal cord tissue caused by the above factors terminates within 8 hours after spinal cord injury (21). In addition, our preliminary data (T. Hashimoto et al., unpublished) have shown that repeated administration of naloxone HCl (45 mg/kg/day, s.c., once daily for 7 consecutive days starting 24 hours after injury), which improves neurologic outcome by acute treatment (22) as TRH does (11, 23), did not show any accelerating effect on the neurologic recovery in rats with spinal cord compression injury. Thus, it is difficult to assume that the acceleration of the neurologic recovery by treatment with TRH starting 24 hours after injury is due to its antagonistic action on these factors, although the ameliorating effect of acutely administered TRH (23) may be due to the mechanisms described previously.

It has been proposed that the increased projection fields due to collateral sprouting participate in the mediation of the functional recovery that occurs following the partial hemisection (24) and deafferentation (25) of the cat spinal cord. Thus, it is conceivable that similar mechanisms mentioned above are involved in the restitution of the neurologic function after spinal cord injury, because some descending pathways remain intact after cord injury (26). In addition, neurotrophic activity of TRH on spinal cord tissue has been reported by several investigators (27, 28). These together suggest that the neurologic recovery-accelerating effect of treatment with TRH starting 24 hours after spinal cord injury would be due to the facilitating action of TRH on the increase in projection fields. This idea has already been proposed by Faden et al. (11).

In conclusion, once or twice daily subcutaneous treatment with TRH starting 24 hours after spinal cord injury for 7 consecutive days dose-dependently accelerated the recovery of neurologic functions, including sensory-motor and urinary bladder functions, in rats with spinal cord injury, and a twice daily dosage schedule tended to produce better improvement in the neurologic state than a once daily schedule when the dose of TRH was intermediate. Although the accelerating effect of TRH on the neurologic recovery after spinal cord injury seems to be attractive, little attempt has been made to clarify the underlying mechanisms. Thus, further studies seem to be needed to explain this effect of TRH.

Acknowledgement: The authors are grateful to Mr. H. Nishikawa for expert technical assistance.

References
8. Matsumoto, A., Kumagai, T., Takeuchi, T.,...


21 Albin, M.S., White, R.J., Yashon, D. and Harris, L.S.: Effects of local cooling on spinal cord trauma. J. Trauma 9, 1000–1008 (1969)


