T-588 Protects Motor Neuron Death Following Axotomy

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R(−)-1-(benzo [b] thiophen-5-yl)-2-[2-(N,N-diethylamino)ethoxy] ethanol hydrochloride) (T-588) enhances acetylcholine release. This compound slows the motor deterioration of wobbler mouse motor neuron disease and enhances neurite outgrowth and choline acetyltransferase activity in cultured rat spinal motor neurons. We examined the ability of T-588 on axotomized spinal motor neuron death in the rat spinal cord. After the postnatal unilateral section of sciatic nerve, there was approximately a 50% survival of motor neurons in the fourth lumbar segment. In comparison with vehicle, intraperitoneal injection of T-588 for 14 consecutive days rescued spinal motor neuron death. Our results showing in vivo neurotrophic activity of T-588 for motor neurons support the applicability of T-588 for the treatment of motor neuron diseases, such as amyotrophic lateral sclerosis and motor neuropathies.

KEY WORDS: T-588; motor neuron; axotomy; amyotrophic lateral sclerosis; motor neuropathies.

INTRODUCTION

Rescue of damaged neurons and stimulation of neurogenesis are theoretically attractive strategies for the treatment of neurodegenerative disorders. The survival of developing motor neurons depends on factors secreted by their muscle targets and by cells in the central nervous system (1).

Several neurotrophic factors have been found to promote motor neuron survival both in vitro and in vivo (2,3). Most of the in vivo studies exploit the fact that lesion of a peripheral nerve in neonatal rodents leads to a rapid and reproducible degeneration of neurons that project their axons into the nerve (4).

Various agents have been studied for their potential efficacy in axotomized motor neuron death. Indeed, axotomy-induced cell death represents a convenient in vivo assay for testing the survival-promoting effects of putative trophic agents on motor neurons.

R(−)-1-(Benzo[b]thiophen-5-yl)-2-[2-(N,N-diethyl amino)ethyl]) ethanol hydrochloride is a novel compound that has been shown to exhibit a wide range of neurotrophic effects both in vivo and in vitro. In mouse spinal motor neurons, T-588 delays the progression of wobbler mouse motoneuron disease (5) It also enhances neurite outgrowth and choline acetyltransferase (ChAT) activity in primary explant culture of ventral spinal cord of fetal rats (6).

The physiological importance of putative motor neuron trophic factors must be experimentally determined in vivo. In this paper, we examined whether T-588 prevents neuronal loss after axotomy by sciatic nerve transection.
in neonatal rats. The degree of neuronal lesion in treated versus control animals was assessed by ChAT-stained paraffin sections of the lumbar enlargement.

EXPERIMENTAL PROCEDURE

We performed sciatic nerve transection in newborn rats, which results in rapid and reproducible death of axotomized motor neurons (7). Left sciatic nerve was transected near the obturator tendon in thigh of newborn Sprague-Dawley rats within 1–3 h after birth. In the experimental group, neonatal rats were injected intraperitoneally with 3, 10, or 30 mg/kg of T-588 daily for 14 consecutive days and the effect of this treatment on the number of spinal motor neurons was assessed. Control rats received an equal volume of phosphate buffered saline (PBS) in the same fashion. For each dose six animals were used. The first injection was performed immediately after axotomy. Two weeks after the nerve section, animals were perfused through the left ventricle with Tyrode solution (room temperature, pH 7.3) followed by a phosphate buffer mixture of 1% paraformaldehyde and 1.5% glutaraldehyde (ice cold, pH 7.4). The lumbar vertebral canal and dura were opened, landmarks such as vertebral bodies and spinal roots identified, and the spinal segment L4 removed (8). The specimen was rinsed in water, dehydrated with increasing concentrations of ethanol (70%–100%), and embedded in paraffin. Serial sections of the spinal cord were cut transversely at 20 μm and stained with ChAT. The number of motor neurons (cell diameter >10 μm) was counted in 10 serial sections on both the operated and contralateral intact side (9). Only those neurons with a distinct nuclear contour containing one or two clear nucleoli were counted under either X125 or X250 (7). The counts were not corrected for split nucleoli. Comparisons of motor neuron counts in the different groups were analyzed statistically by Student’s t test. All data are expressed as mean ± SEM.

RESULTS

Animals tolerated the T-588 treatment well. The number of spinal motor neurons per slice was reduced to approximately half that of nonaxotomized motor neurons in vehicle-treated rats. At 3 mg/kg of T-588 there was no significant difference in the number of motor neurons per slice in the axotomy side compared to that of the control group. T-588 10 and 30 mg/kg significantly attenuated the loss of motor neurons in the axotomy side. There was no significant difference for the motor neuron numbers between 10 mg and 30 mg/kg in the T-588-treated group. In addition, there was no significant difference for the number of spinal motor neurons in the nonaxotomy side between the experimental and control groups (Figs. 1 and 2). Survival ratio (operated/ nonoperated side × 100) was given as an index of neuronal survival. Of 10 and 30 mg/kg of T-588 significantly

![Graph](image-url)  
**Fig. 1.** Number of motor neurons per slice. T-588 with 10 and 30 mg/kg had significant preservation of motor neuron numbers in axotomy side compared to those with control.