

## Original Article

# Deprenyl Can Mediate Neuronal Protection Rather than Neuronal Rescue

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### ABSTRACT

**Background and Objective:** Deprenyl is a drug for the treatment of Parkinson's disease, where the dopaminergic neurons are the target of this drug. Several reports also documented that deprenyl has an effect on the sensory and motor neurons. There are some reports about the mode of action of deprenyl on motoneurons as a neuroprotective agent, while others believe that deprenyl acts as a neurorescuer.

**Materials and Methods:** In this experimental study, the axotomized spinal motoneurons in rat neonates were used to investigate the mode of action of deprenyl on motoneurons. Six groups of newborn rats (5 each) were used in this study. The first group was treated with 2.5 mg/kg of drug (for 21 days) one hour before surgical transection of the left sciatic side, the second treated at the time of surgery, and the third one treated one hour after surgery. The fourth, fifth, and sixth groups were given normal saline 1 hour before the surgery, at the time of surgery, and 1 hr after the surgery respectively. The animals were perfused and spinal cords were removed. The tissues were processed in paraffin and then sectioned. Tissues were stained with Cresyl violet. Total motoneuron count was done and the percentage of motoneuron reduction as well as motoneuron survival index was calculated.

**Results:** The obtained data revealed that deprenyl in pre-treated group was more effective than in the other two modes of treatment.

**Conclusion:** Taken together, deprenyl is more neuroprotective than neurorescuer of spinal motoneurons in rats.

**Key words:** Deprenyl, Spinal cord, Neuron

### Introduction

Deprenyl is a type B monoamine oxidase inhibitor which is used to treat Parkinson's disease (1), where deprenyl showed a direct effect on dopaminergic neurons (2). The anti-Parkinson's disease activity of the drug has been demonstrated in animal model (3) and in clinical studies (4). Several mechanisms have

been proposed for the mode of action of deprenyl on dopaminergic neurons including direct inhibition of MAO-B and subsequent increase in the level of dopamine in these neurons (2). The mechanisms of anti-oxidant activities were suggested directly by free-radical scavenging (5, 6), or indirectly by induction of free radical scavenging enzymes such as superoxide dismutase (7).

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Received: 10 February 2007

Accepted: 25 April 2007

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On the other hand, deprenyl-treated axotomized-motoneurons in the spinal cord of newborn rats showed a sustained increase in the number of the cells as compared with those untreated newborns (8). In vitro findings have confirmed these results (7, 9). The axotomized adult murine facial motoneurons showed a significant increase in the number of neurons at the axotomized motoneuron treated with deprenyl as compared with those untreated ones (10). The investigation on the sensory neurons revealed similar findings in dorsal root ganglion where the number of the neurons in the transected animals treated with deprenyl showed higher neuronal number in comparison with untreated ones (11). Also, Buys et al. (12) reported that deprenyl increases the survival of rat retinal ganglion cells following optic nerve crush. These observations indicated that deprenyl can increase the survival of neurons after trauma. While, some investigators proposed that deprenyl protects the axotomized motoneurons (8, 13) and others assumed that deprenyl rescues these neurons (14, 15, 16).

Therefore, the purpose of this study was to elaborate the mode of deprenyl action regarding neuroprotection versus neurorescue of axotomized motoneurons.

### Materials and Methods

Sprague-Dawley rats were purchased from Razi Institute (Karaj, Iran), kept in the Shahed University animal house, and handled according to the guidelines of the university ethical committee. Six groups (5 each) of newborn rats were used in the study, left sciatic nerves were transected while the right ones kept intact and used as controls. The animals were anesthetized by hypothermia and the surgery was done on day 3 postnatal. The first group was treated daily with deprenyl 2/5 mg/kg one hour before the surgery by intraperitoneal injection, the second group was treated at the surgery time, and the third group was treated one hour after the surgery. The treated rats were maintained for 21 days. The animals were sacrificed using general anesthesia. Accordingly, the fourth, fifth, and sixth groups were injected with normal saline similar to the protocols of the treated animals. The L4-L6 spinal segments were removed by laminectomy and perfused with Karnivasky` fixative and then in buffered formalin, processed in paraffin, cut (8 mm), and stained with Cresyl violet.

Motoneurons in axotomized and intact sides were counted, one from five sections in each slide was counted and then according to Panahi and Al-Tiraihi (8) total motoneurons calculated, and the percentage

of motoneuron reduction was also calculated (8). The therapeutic effectiveness was calculated as follows:

Motoneuron survival index = PMR in the saline treated group/PMR in the deprenyl treated group.

PMR is the percentage of motoneuron reduction.

The data were tested for normality using S-K test and analyzed using Student's t-test and analysis of variance.

### Results

The results showed that the total number of motoneurons in deprenyl treated group at the axotomized side was significantly higher than those of untreated animals. Total number of motoneurons in intact side (control) was higher than axotomized side in all 6 groups. A similar trend was noticed in the percentage of motoneuron reduction (Table 1). The percentage of motoneurons reduction in pre-surgery treatment group was significantly lower than the post-surgery treated groups, while it was insignificantly lower than those treated at surgery. The motoneuron survival index in the pre-surgery treatment group (1.7) was higher than those treated at surgery time and post-surgery treatment group (1.6 and 1.4 respectively).

**Table1. The means and standard deviations of total motoneurons count (TMC) at the axotomized (ax) and un-axotomized (un) sides in pre-surgery treatment (1 h), treated at surgery time, and post-surgery treated (1 h) were presented as well as the percentage of neuronal reduction (PNR) (means and standard deviations) and motoneuron survival index (MSI) (ratio) in the same groups.**

Ux and ax denote un-axotomized and axotomized group, D and S indicate deprenyl and saline normal-treated groups respectively.

Parameters	Pre-surgery treatment	At surgery treatment	Post-surgery treatment
TMC-ux-D	1577±79*	1672±72 *	1844±194*
TMC-ax-D	1071±90*	1111±117*	1105±148*
TMC-ux-S	1751±150•	1903±98•	1820±184•
TMC-ax-S	800±125•	817±142•	865±106•
PNR-D	-35.5±2.4	-38.2±4.7	-39.6±2.6
PNR-S	-54.8±5	-54.7±5.4	-55.2±4.5
MSI	1.7	1.6	1.4

The statistical differences in TMC between the axotomized and un-axotomized sides were significant in all groups (Treated group \*) (untreated group •).

The statistical differences in TMC between the un-axotomized sides in pre-surgery, at-surgery, and post-surgery in treatment groups and non- treated groups were not significant, while in the axotomized sides, the statistical differences were significant between pre-surgery with post and at surgery in treatment groups.

The differences in PNR between pre-surgery and post and at-surgery in treatment groups were significant. The statistical differences in PNR between the pre, post, and at surgery in saline normal treatment groups were not significant.

## Discussion

The results of this study showed that deprenyl reduces the motoneuron loss following axotomy which is consistent with the findings of other investigators (14, 17, 18). In this report we were able to show that deprenyl can protect the axotomized motoneurons from death and did not lead them to apoptotic phase, so deprenyl act as a neuroprotective agent rather than a neurorescuer. Similar to this, other investigations have reported that pre-surgery treatment with deprenyl protect the dopaminergic neurons in an animal model of Parkinson's disease (19). Neuroprotective effects of deprenyl against the effect of neurotoxins have been reported too (3). This may be the effect of deprenyl on the free radical agent or up-regulates superoxide dismutase and catalase, and suppresses non-enzymatic and iron-catalyzed auto-oxidation of dopamine (17, 20). On the other hand, a neurorescuer role was proposed for the action of deprenyl in the reduction of dopaminergic neuron death following 1-methyl-4-phenyl-1, 2, 3,6 tetrahydropyridine neurotoxin conversion into MPP<sup>+</sup> that confirmed the neurorescue mechanism of dopaminergic neurons (3, 19).

The use of post-surgery treatment was also reported to protect these neurons using the same neurotoxin model. Sziraki et al (21) and Thiffault et al (22) reported similar results in mice injected with MPTP. However, pre-surgery treatment with deprenyl confirmed neuroprotection effect in the same model (23). Moreover, pre-surgery treatment with deprenyl has been reported to protect PC12 cells in the serum and NGF for 24 h (18).

Neuroprotection of motoneurons can not exclude the possibility of the neurorescue mechanism, because most of the previous studies used post-surgery treatment

protocol in axotomized spinal motoneurons (8), injured motoneurons due to spinal cord ischemia (24), and axotomized facial motoneurons (10). In this study, pre-surgery treatment protocol was used to evaluate the neuronal loss in the axotomized motoneurons and the results showed that there is a statistically significant increase in the number of motoneurons in the pre-surgery treatment group at the axotomized side (Table 1). However, the mechanism of neuroprotection by deprenyl in post-surgery treatment protocol can not be excluded because of the existence of delayed phase of motoneurons death in the injured spinal cord (21).

## Conclusion

The conclusion of this study is that deprenyl is more neuroprotective than Neurorescue of spinal motoneurons in rat animal model.

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