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Short communication

β-Aminopropionitrile treatment can accelerate recovery of mice after spinal cord injury

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Abstract

Modulations of the extracellular matrix and scar formation following central nervous system (CNS) injuries are considered prohibitive for axon regeneration, thus restricting functional recovery. Recent findings indicating that lysyl oxidase, an extracellular matrix-forming enzyme, appears in a time-dependent manner at brain injury sites have suggested that inhibition of this enzyme may be conducive for regeneration and functional recovery. Here, we report that after unilateral spinal cord transection in adult mice, daily treatment (for 20 days) with the lysyl oxidase inhibitor β-aminopropionitrile (100 mg/kg intraperitoneal) resulted in accelerated and more complete functional recovery. The mode of functional recovery, however, indicates that axonal regeneration of long descending tracts did not occur.

Keywords: Regeneration; Extracellular matrix; Lysyl oxidase; Sensorimotor function

1. Introduction

Neurons in the adult mammalian central nervous system (CNS) are incapable of successful regeneration following injury of their axon. Initial regenerative efforts are aborted resulting in a permanent loss of bodily functions (Cajal, 1928). This failure of regeneration is attributed in large part to inimical factors in the local extracellular environment (Bignami et al., 1988; Schwab, 1996; Stichel and Muller, 1998). Lysyl oxidase (EC1.4.3.13) is a secreted enzyme that catalyzes crosslinking of extracellular matrix proteins (Kagan, 1994). Recently, we found evidence, that early after CNS injury active extracellular lysyl oxidase molecules accumulate in a spatiotemporal manner in the lesion site and, as indicated by Northern blot analysis, cells in the region of injury synthesize this enzyme (Gilad et al., 2000). Based on these findings, we hypothesized that lysyl oxidase is involved in modulating the extracellular matrix and in scar formation at CNS injury sites, thus playing an unwanted role in wound healing with lasting implications for functional recovery, and that its inhibition will be beneficial for recovery of function. β-Aminopropionitrile, an active site irreversible inhibitor of lysyl oxidase (Kagan, 1994), is known to alter the physical characteristics of scar tissue and as such was previously suggested to have potential in reducing the physical barrier to regeneration in the injured spinal cord (Rankin et al., 1983). In the present study, therefore, we treated adult mice with β-aminopropionitrile after unilateral spinal cord transection and measured the rate of functional recovery from sensorimotor and locomotor deficits.

2. Methods

Two-month-old male BALB/c mice, kept under the Institute's Animal Care and Use Committee approved protocols, were anesthetized with halothane (1.5% in 100% O_2). Following laminectomy, the spinal cord was injured by complete unilateral transection at the T10–T11 level with a sharp knife as previously described (Gilad et al., 1979). This injury model results in acute ipsilateral sensorimotor paralysis that recovers to a large extent over time, with the contralateral hind limb remaining intact (Gilad et al., 1979). Mice were randomly assigned to two groups, one was injected (intraperitoneally) daily (1st injection at 10 min postoperative) for 20 days with 100 mg/kg β-aminopropionitrile (monofumarate, Sigma-Aldrich, Israel) and the other group was injected with vehicle (saline, 0.9% NaCl). The two groups were compared for the rate of

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recovery from sensorimotor deficits as assessed by a five-component test battery: (I) A 17-point locomotor scale modified (for unilateral injury in mice) from Basso et al. (1995) as outlined in Table 1. (II) Limb withdrawal reflex (extension and pain stimuli). (III) Placing reflex (the contact placing response was elicited by rubbing the dorsal and lateral aspect of the foot). (IV) Toe spread reflex. Tests II, III and IV were performed and graded by scales modified from Von Euler et al. (1996) as follows: 0—no response, 1—weak response, 2—normal response. And (V) "Tail-pull test", whereby, after recovery from the initial ipsilateral hind limb flaccid paralysis and contralateral bent of the trunk, ipsilateral hind limb spasticity is examined in animals trying to escape when being pulled by the tail (Gilad et al., 1979). Ipsilateral spasticity indicates interruption of the corresponding long descending tracts and no recovery is expected unless regeneration has occurred. "Pre-lesion" values were recorded for each animal before infliction of the injury.

Table 1
Ipsilateral hind limb locomotor rating scale following unilateral spinal cord hemisection in mice

Score	Motor function
0	No movement
1	Slight movement of one or two upper joints
2	Slight movement of two or all three joints
3	Slight movement of all three joints
4	Extensive movement of the upper joint with slight movement of two joints
5	Extensive movement of two upper joints with slight movement of the third (lower) joint
6	Extensive movement of all three joints
7	Sweeping movement, no weight support
8	Dorsal or plantar stepping with weight support
9	Occasional (0–50% of the time) weight-supported plantar stepping, no gait coordination with contralateral limb
10	Weight-supported plantar stepping, no coordination
11	Occasional weight-supported plantar stepping, occasional coordination
12	Weight-supported plantar stepping, frequent (50–95% of the time) coordination, occasional toe clearance
13	Weight-supported plantar stepping, consistent (95–100% of the time) coordination, occasional toe clearance
14	Consistent plantar stepping, consistent coordination, frequent toe clearance, contralateral bent trunk
15	Consistent plantar stepping, consistent coordination, consistent toe clearance, contralateral bent trunk
16	Consistent plantar stepping, consistent coordination, consistent toe clearance, evenly held trunk
17	Normal gait

One hundred and twenty days after the operation, sensorimotor functions were examined before and after production of a second identical spinal cord hemisection (at the original injury site) (Gilad et al., 1979). This "re-lesion" was performed in order to verify whether the observed recovery was due to regeneration of the cut axonal tracts (Gilad and Kupmar, 1982; Gilad et al., 1979).

3. Results

As illustrated in Fig. 1A, mice treated with β-aminopropionitrile showed an accelerated and more complete recovery of locomotor deficits when compared with saline-treated controls. There were no substantial differences in the recovery rate of the placing reflex (Fig. 1B). But, the severity of initial deficits was less and the recovery rate of limb withdrawal reflexes was faster during the first 5 postoperative days in β-aminopropionitrile-treated mice (Fig. 1C and D). The toe spread reflex was abolished after injury and never recovered in either group (results not shown).

Recovery from ipsilateral hind limb flaccid paralysis was complete after a similar time interval in both groups (6.00 ± 0.63 and 5.66 ± 0.25 days in saline- and β-aminopropionitrile-treated mice, respectively), the time when full ipsilateral spasticity in the tail-pull test ensued. The ipsilateral hind limb remained spastic in both groups for up to 120 days after the injury.

Ipsilateral hind limb flaccid paralysis did not occur in either group after the second identical transection made 120 days after the first injury. Instead, ipsilateral hind limb spasticity was observed in animals of both groups immediately after re-lesioning. At 24 h after re-lesioning, placing and withdrawal reflex scores did not decline appreciably in animals of both groups (Fig. 1B,C, and D), while locomotor scores were significantly reduced by 40% and 25% in saline- and β-aminopropionitrile-treated groups, respectively (Fig. 1A).

4. Discussion

The data clearly demonstrate that β-aminopropionitrile treatment of adult mice after unilateral spinal cord transection can result in accelerated recovery of sensorimotor functions and lead to a more complete recovery of locomotor functions of the ipsilateral hind limb. The immediate and early beneficial effects of β-aminopropionitrile treatment on sensorimotor functional deficits result most probably from inhibition of molecular processes that cause secondary damage at the site of spinal cord injury. Reduction of secondary damage would limit expansion of the lesion and, in turn, lead to the long-lasting beneficial effects of treatment. Several parameters, however, indicate

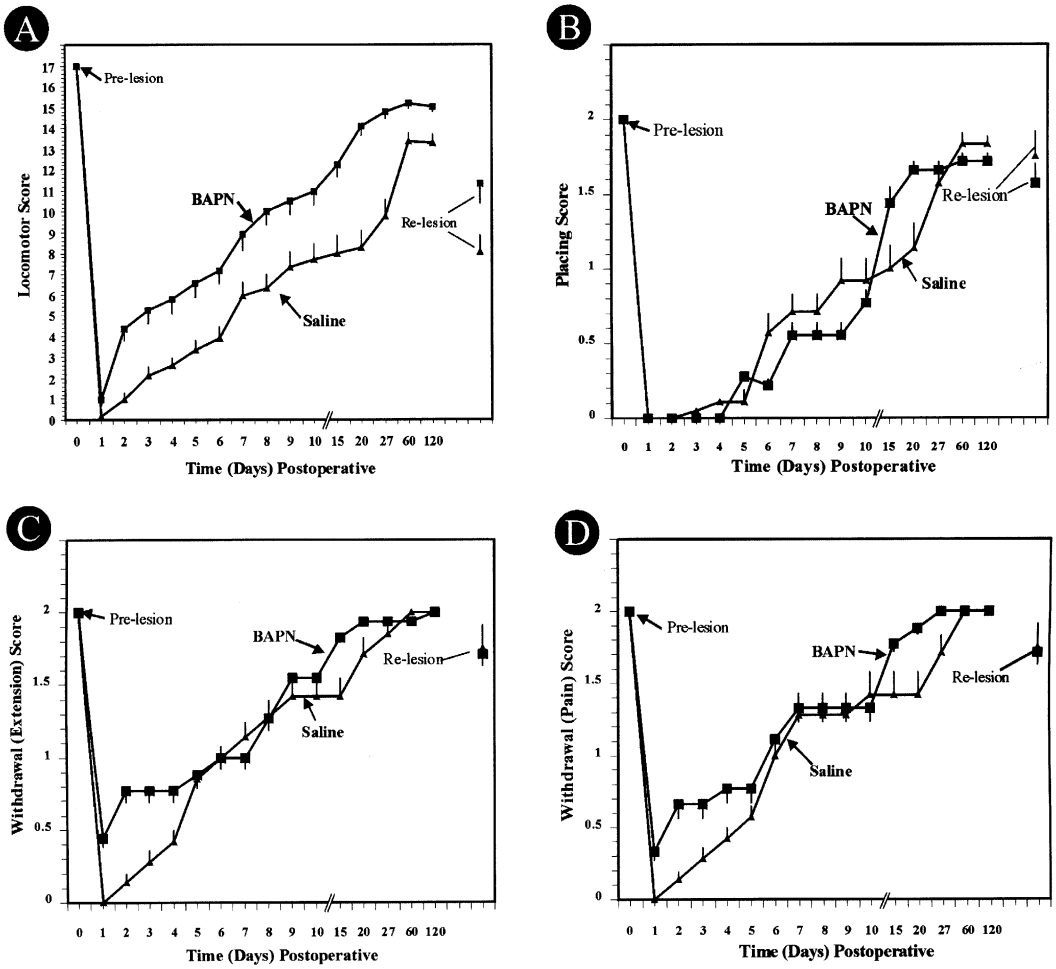

Fig. 1. Effects of β-aminopropionitrile (BAPN) (■) or saline (▲) treatment on: (A) Locomotion; (B) placing reflex; (C) withdrawal reflex to extension stimulus; and (D) withdrawal reflex to pain stimuli, following unilateral spinal cord transection. Results are the mean (±S.D., vertical lines) values for 10 animals in each group. Note the interrupted scale (in days) of the abscissa. Differences between groups were statistically significant ($P \leq 0.01$ using two-tailed *t*-test) at the following postoperative times: (A) at all time points after 1 day postoperative; (B) at 6, 9, 15 and 20 days postoperative; (C) at 1–4 and 15–20 days postoperative; and (D) at 1–4 and 15–27 days postoperative.

that the mode of the observed long-lasting functional recovery was not the result of successful axonal regeneration of long descending spinal cord fiber tracts: (1) The early ensuing spasticity was persistent. (2) The immediately lost toe spread reflex failed to recover. And (3) Flaccid paralysis did not recur, while sensorimotor functions and spasticity persisted after re-lesion (i.e., after the second identical spinal cord transection at the original site of injury).

Spasticity due to hyperreflexia is a common consequence of spinal cord injury in animals and humans (Burke, 1988; Thompson et al., 1992). It occurs as a result of synaptic disinhibition after severance of the inhibitory descending cortical and brain stem tracts (Andersen et al., 1962). Thus, the observations that spasticity remained and that re-lesion had minimal effects indicate that the mode of the observed functional recovery is most probably due to plastic synaptic changes in the spinal cord distal to the unilateral transection. How treatment with β-aminopropionitrile, a known inhibitor of lysyl oxidase activity, augments this mode of sensorimotor recovery remains to be elucidated. Our recent findings showed that early (within 1 day) after brain injury in rats, lysyl oxidase immunoreactivity accumulates at the lesion site and that enzyme activity is transiently increased over a period of 20 days (with a peak at 10 days) in the injury region (Gilad et al., 2000). Thus, if, as we hypothesized, β-aminopropionitrile inhibits lysyl oxidase activity during this critical period, it may prevent or delay unwanted modulation of the extracellular matrix, and lysyl oxidase inhibition should be considered a target for pharmacological treatment of spinal cord injury.

Still, one should consider the possibility that systemic β-aminopropionitrile treatment may somehow affect the outcome of injury indirectly via lysyl oxidase inhibition and extracellular matrix modulation in tissues other than the spinal cord. And, while the action of β-aminopropionitrile on molecular targets other than lysyl oxidase is

uncertain at this time, this possibility should not be excluded. In this regard, it is interesting to note that topical application of β-aminopropionitrile was reported to modulate evoked potential conduction in rat sciatic nerve and spinal cord (Gunasekaran et al., 1987). β-Aminopropionitrile is known to induce pathological changes in bone (osteolathyrism) and blood vessels (angiolathyrism) (Spencer and Schaumburg, 1983). And, while β-aminopropionitrile may not be considered neurotoxic (Spencer and Schaumburg, 1983; Denlinger et al., 1994), purkinje cell toxicity was observed in rats after high-dose treatment (1 g/kg) over an extended period (10 weeks) (Martin et al., 1991), and toxic changes were observed after addition of β-aminopropionitrile to neuronal cultures (Capo et al., 1994).

In summary, the study shows that β-aminopropionitrile treatment can result in an accelerated and more complete functional recovery after spinal cord injury in adult mice and indicates that lysyl oxidase inhibition may be considered a therapeutic target after spinal cord injury.

References

Andersen, P., Eccles, J.C., Sears, T.A., 1962. Presynaptic inhibitory action of cerebral cortex on the spinal cord. Nature (London) 194, 740–741.

Basso, D.M., Beattie, M.S., Bresnahan, A., 1995. A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12, 1–21.

Bignami, A., Dahl, D., Gilad, V.H., Gilad, G.M., 1988. Do white matter astrocytes produce a nonpermissive (repellent) substrate for axonal growth? Exp. Neurol. 100, 253–256.

Burke, D., 1988. Spasticity as an adaptation to pyramidal tract injury. Adv. Neurol. 47, 401–423.

Cajal, S.R.Y., 1928. In: May, R.M. (Ed.), Degeneration and Regeneration of the Nervous System. Oxford Univ. Press, New York, NY, pp. 484–760, translated ed.

Capo, M.A., Sevil, B., Frejo, M.T., Anadon-Bselga, M.J., Alonso, C.E., 1994. In vitro neuronal changes induced by beta-aminopropionitrile. J. Environ. Pathol., Toxicol. Oncol. 13, 259–264.

Denlinger, R.H., Anthony, D.C., Amarnath, K., Amarnath, V., Graham, D.G., 1994. Metabolizm of 3, 3′-iminodipropionitrile and deuterium-substituted analogs: potential mechanism of detoxification and activation. Toxicol. Appl. Pharmacol. 124, 59–66.

Gilad, G.M., Kupmar, V., 1982. Absence of functional recovery after spinal hemisections in mice genetically deficient in hemolytic complement. Exp. Neurol. 76, 666–669.

Gilad, G.M., Gilad, V.H., Kopin, I.J., 1979. Reaction of the mutant mouse *nude* to axonal injuries of the central nervous system. Exp. Neurol. 65, 87–98.

Gilad, G.M., Malcov, M., Kagan, H.M., Gilad, V.H., 2000. The extracellular matrix enzyme lysyl oxidase in CNS injury sites. Soc. Neurosci. USA Abstr. 26 (1), 857.

Gunasekaran, S., Weinstein, P., Chvapil, M., 1987. Effect of topical beta-APN application on evoked potential conduction in rat sciatic nerve and spinal cord. Surg. Neurol. 28, 201–207.

Kagan, H.M., 1994. Lysyl oxidase: mechanism, regulation and relationship to liver fibrosis. Pathol., Res. Pract. 190, 910–919.

Martin, J.E., Sosa-Melgarejo, J.A., Swash, M., Mather, K., Leigh, P.N., Berry, C.L., 1991. Purkinje cell toxicity of beta-aminopropionitrile in the rat. Virchows Arch. A: Pathol. Anat. Histopathol. 419, 403–408.

Rankin, L.L., Chvapil, M., Misiorovsky, R., Johnson, P., Weinstein, P.R., 1983. Diffusion characteristics of beta-aminopropionitrile in peripheral nerve. Exp. Neurol. 79, 97–105.

Schwab, M.E., 1996. Molecules inhibiting neurite growth: a mini review. Neurochem. Res. 21, 755–761.

Spencer, P.S., Schaumburg, H.H., 1983. Lathyrism: a neurotoxic disease. Neurobehav. Toxicol. Teratol. 5, 625–629.

Stichel, C.C., Muller, H.W., 1998. The CNS lesion scar: new vistas on an old regeneration barrier. Cell Tissue Res. 294, 1–9.

Thompson, F.J., Reir, P.J., Lucas, C.C., Parmer, R., 1992. Altered patterns of reflex excitability subsequent to contusion injury of the rat spinal cord. J. Neurophysiol. 68, 1473–1486.

Von Euler, M., Akesson, E., Samuelsson, E.B., Seiger, A., Sundstrom, E., 1996. Motor performance score: a new algorithm for accurate behavioral testing of spinal cord injury in rats. Exp. Neurol. 137, 242–254.