

## Effect of aluminum on neurological recovery in rats following spinal cord injury

**KHALAF AL MOUTAERY, F.R.C.S., SALEH AL DEEB, F.R.C.P., NABIL BIARY, M.D., CHRISTUDAS MORAIS, M.PHIL., HASEEB AHMAD KHAN, PH.D., AND MOHAMMAD TARIQ, F.R.C.PATH.**

*Neuroscience Research Group, Armed Forces Hospital, Riyadh, Saudi Arabia*

**Object.** This investigation was undertaken to study the effect of aluminum on neurobehavioral, electrophysiological, structural, and biochemical changes in rats following spinal cord injury (SCI).

**Methods.** Adult male Sprague–Dawley rats classified into different groups were given aluminum sulfate–dosed drinking water in the concentrations of 0%, 0.25%, 0.5% and 1%, respectively. After 30 days of aluminum treatment, the animals were subjected to spinal cord trauma. Laminectomy was performed at T7–8 in anesthetized rats, followed by placement of a compression plate (2.2 × 5 mm) loaded with a 35-g weight over the exposed spinal cord for 5 minutes. Control animals underwent the same surgical procedure, but the compression injury was not induced (sham). Postoperative neurological function was assessed using the inclined-plane test and by obtaining a modified Tarlov score and vocal/sensory score daily for 10 days. Electrophysiological changes were assessed using corticomotor evoked potentials, whereas pathological changes were assessed by light microscopy. The level of vitamin E in the spinal cord was measured as an index of antioxidant defense. The behavioral, biochemical, and histological analyses were performed in a blinded fashion.

**Conclusions.** Analysis of results obtained in the behavioral studies revealed that the compression of spinal cord produced transient paraparesis in which a maximum motor deficit occurred at Day 1 following SCI and resolved over a period of 10 days. Administration of aluminum significantly impaired the recovery following SCI. Analysis of the results of the biochemical, electrophysiological, and histopathological studies also confirmed the deleterious effects of aluminum on recovery from SCI in rats.

**KEY WORDS** • experimental spinal cord injury • aluminum • vitamin E • electrophysiology • rat

**S**PINAL cord injury is considered to be a major cause of disability and has many physical, psychological, and social ramifications.<sup>14</sup> The main causes of traumatic SCI are motor vehicle accidents, sports and recreational activities, accidents at work, and falls at home.<sup>72</sup> In clinical and experimental studies it has been shown that after traumatic SCI a series of pathophysiological events occur in and around the injured tissue that may lead to partial disability or complete paralysis.<sup>65</sup> It has been hypothesized that there are two mechanisms that play important roles following SCI. The primary injury mechanism includes acute compression, laceration, destruction, and shear of spinal cord tissue.<sup>13,30,62</sup> Allen<sup>4</sup> was the first to describe the concept of secondary injury following neurotrauma. According to this theory, the primary insult may lead to a variety of secondary changes that cause destruction of spinal cord as a result of vascular changes, loss of autoregulation, systemic hypotension, hemorrhage, thrombosis, and vasospasm.<sup>73</sup> The series of biochemical, pathological, and immunological events that occur in the

period of hours to days following initial injury might be more hazardous than the damage caused by primary impact.<sup>29</sup> It has been shown in recent studies that any pre-existing disease or concomitant exposure to toxic chemicals may significantly impair recovery from neurotraumatic injury.<sup>2,28,31,44,68</sup>

Since the first weight-drop model developed by Allen<sup>4</sup> in 1911, several experimental models based on compressive injury have been designed to simulate human SCI.<sup>50,59,71</sup> Although Allen's model has the advantage of its dynamic similarity to the clinical situation found in human SCI, its major shortcoming is the lack of reproducibility.<sup>73</sup> The static-load technique introduced by Nystrom, et al.,<sup>50</sup> produces reproducible injury: the combined contusive and ischemic lesions mimic the compressive injury found in spinal cord injured–patients in whom vertebral body dislocation and cervical wedge fractures have occurred.<sup>50,80</sup> In traumatic SCIs in humans, the chance of therapeutic success is greatest among those who have sustained an incomplete injury.<sup>50</sup> The static-load compression model, which produces an incomplete injury followed by a well-defined recovery pattern, has been used extensively for pharmacological intervention studies in the recent years.<sup>2,55,67,68,77</sup>

*Abbreviations used in this paper:* CMEP = corticomotor evoked potential; CNS = central nervous system; SCI = spinal cord injury.

## Aluminum exacerbates spinal trauma

Aluminum is the third most abundant element (accounting for 8%) in the earth's crust. The major sources of aluminum exposure include the use of aluminum salts in municipal water treatment plants,<sup>79</sup> use of aluminum utensils,<sup>42</sup> aluminum-containing phosphate binders,<sup>66</sup> antacids,<sup>58</sup> toothpastes,<sup>75</sup> and consumption of aluminum-contaminated beverages<sup>45</sup> and nutrient solutions<sup>39</sup> and, accidental spills of aluminum salts in water.<sup>5</sup> Its concentration in water varies from a few tenths to hundreds of micrograms per liter.<sup>24</sup> The daily dietary intake of aluminum in humans from food and water ranges between 3 mg and 100 mg,<sup>27</sup> and a serum aluminum level up to 10 µg/L is considered safe.<sup>20</sup> Ingestion of aluminum-containing antacids would cause an additional exposure of approximately 5 g of aluminum per day,<sup>43</sup> which might lead to a 10-fold increase in serum aluminum in an individual with normal kidney function.<sup>49</sup> On the other hand, use of antacids or phosphate-binders in patients with impaired renal function has been shown to cause a 40- to 100-fold increase in these serum levels.<sup>8,76</sup> The increase in aluminum body burden may lead to neurotoxicity, nephrotoxicity, osteodystrophy, and hypochromic anemia.<sup>6,22,48</sup> Taking into account the aforementioned facts, the present investigation was undertaken to study the effect of aluminum on neurological recovery following SCI in rats.

### Materials and Methods

#### *Animals and Drugs*

Adult male Sprague-Dawley rats weighing 180 to 200 g were housed in a temperature-controlled room and maintained in a 12-hour light/dark cycle. Standard laboratory animal food and aluminum-dosed drinking water were freely available throughout the study. Four treatment groups (eight rats each) were treated with 0%, 0.25%, 0.50%, and 1% of aluminum sulfate ( $\text{Al}_2[\text{SO}_4]_3 \cdot 18 \text{H}_2\text{O}$ ) in drinking water, respectively, for 30 days before being subjected to SCI. The selected doses were similar to those used in several recently published studies.<sup>1,16,26,69</sup> These doses are equivalent to 0 mg, 375 mg, 750 mg, and 1500 mg of aluminum per day consumed by humans weighing 70 kg and are similar to the amounts usually present in antacids ingested by people with peptic disorders. Recently, we observed that administration of 0.25 to 1% of aluminum sulfate in drinking water produced a several-fold increase in serum aluminum levels in rats.<sup>69</sup> All the experiments in the present study were undertaken according to the guidelines provided by the Research and Ethical Committee of Armed Forces Hospital, Riyadh, Saudi Arabia.

#### *Spinal Cord Injury*

After 30 days of aluminum treatment, the animals were subjected to spinal trauma according to the method described by Nystrom and Berglund.<sup>51</sup> The animals received an anesthetic of chloral hydrate (400 mg/kg subcutaneously) and a T7-8 laminectomy was performed, leaving the dura intact. A slightly curved rectangular compression plate ( $2.2 \times 5$  mm) loaded with a weight of 35 g was gently placed over the exposed spinal cord for 5 minutes. The wound was closed in layers, and the animal was allowed to recover from anesthesia. Animals in the control group underwent the same surgical procedure but without receiving any compression injury (sham treatment). All the animals received intramuscular injection of gentamicin (2 mg/kg) daily for 3 days after surgery. The bladder was pressed manually twice a day to avoid urinary complications.

#### *Behavioral Studies*

Neurological function following SCI was assessed daily for 10 days by obtaining a modified Tarlov score,<sup>70</sup> a Rivlin and Tator

angled-plane score,<sup>60</sup> and a vocal/sensory score.<sup>10</sup> A modified Tarlov score was used to assess the hindlimb function as follows: 0 = total paraplegia of hindlimbs; 1 = no spontaneous movement but responds to hindlimb pinch; 2 = spontaneous movement; 3 = able to support weight but not able to walk; 4 = walks with gross deficit; 5 = walks with mild deficit on broad flat surface; 6 = able to walk on broad, flat surface and support weight on a 1.8-cm-wide ledge; and 7 = walks on ledge.

The angled-plane test consisted of measuring the maximum angle at which an animal can support its weight on an inclined board measured in degrees (0–90°). The board was covered with a rubber mat consisting of 1-mm-high ridges. The animals were placed transversely on the inclined plane, and the highest angle the animal maintained for 5 seconds was recorded and described as the "capacity angle." For every test session three separate measurements were made, and the mean score was determined.

The sensory/vocal score was measured by administering a noxious stimulus (pinching with a toothed forceps) to the hindlimb of the animals.<sup>10</sup> The response of the hindlimb to stimulus was graded as follows: 0 = no response; 1 = withdrawal from pinch without vocalization; 2 = vocalization without withdrawal; and 3 = vocalization and withdrawal. For biochemical and histological studies, eight additional animals from each group were killed at 4 hours, 24 hours, 5 days, and 10 days post-SCI. All the behavioral, biochemical, and histological tests were performed in blinded fashion.

#### *Analysis of $\alpha$ -Tocopherol in Spinal Cord Tissue*

The level of  $\alpha$ -tocopherol (vitamin E) in spinal cord tissue was analyzed using high-performance liquid chromatography according to the method described by Dexter, et al.<sup>19</sup> The injured site of the spinal cord (a 50-mg sample) was homogenized in a tube containing 1 ml of Tris buffer (50 mM, pH 7.6) and 3 ml of 1.5% ethanolic pyrogallol. The homogenate was incubated at 70°C in a water bath for 5 minutes, 150 µl of 10 M potassium hydroxide was added to each tube, and additional incubation (70°C) occurred for 30 minutes. The mixture was cooled in an ice bath to room temperature and extracted with 2 ml of hexane. The organic layer was separated after centrifugation, and the aqueous homogenate was further extracted with another 2 ml of hexane. The two hexane extracts were combined and evaporated in nitrogen and stored at –70°C for future analysis.

The high-performance liquid chromatography instrument (Waters Associates Inc., Medford, MA) consisted of a solvent delivery pump (Model 510), autoinjector (Model 712), UV-Visible detector (Model 481), and integrator (Model 740). The column used was Bondapak C-18,  $3.9 \times 150$  mm (Waters Associates, Inc.), made of stainless steel. The mobile phase consisted of 95% chromatography-grade methanol in deionized water. The flow rate of the mobile phase was adjusted to 1.5 ml/minute, and the absorbance was measured at 280 nm following a 60-µl injection. The level of vitamin E was calculated using a calibration curve.

#### *Electrophysiological Monitoring*

Corticomotor evoked potentials were measured using an electromyography system (MS92; Medelec, England) in which the active disc electrode was placed epidurally over the right motor cortex area, whereas the reference needle electrode was placed over the nasion of the anesthetized (400-mg/kg chloral hydrate) rat. A grounding electrode was placed into the skin of the back. Two needle electrodes were placed into the lateral muscle mass of the left hindlimb and foot pad, respectively, to apply electrical stimulation. Transcortical stimulation was achieved by applying a constant square wave pulse of 0.1 msec in duration at 1 Hz at an intensity of 50 V (the stimulus was amplified and filtered in the range of 10–500 Hz). The time base was set at 50 msec.

#### *Histological Examination*

The rats were anesthetized with ether, and intracardiac perfusion was performed with isotonic saline followed by 10% neutral buffered formalin. After perfusion, the injured portion of the spinal cord was removed, embedded in paraffin, and 6-µm sections were

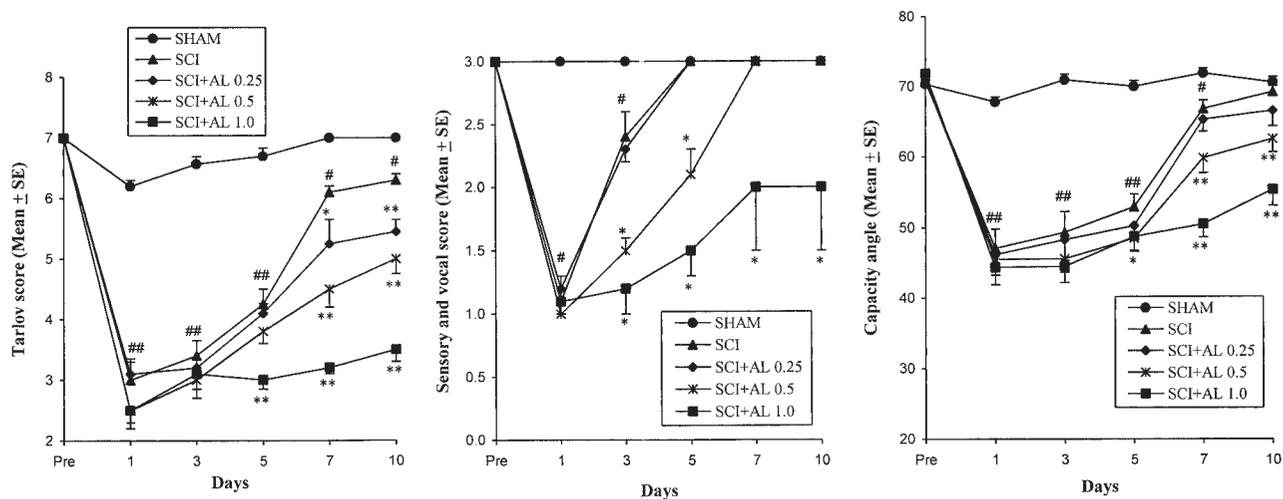


FIG. 1. *Left*: Graph showing the effect of aluminum (AL) on Tarlov score in spinal cord-injured rats. # $p < 0.05$ ; ## $p < 0.01$  compared with SHAM and \* $p < 0.05$ ; \*\* $p < 0.01$  compared with SCI group. *Center*: Effect of aluminum on sensory and vocal score in spinal cord-injured rats. # $p < 0.01$  compared with SHAM; \* $p < 0.01$  compared with SCI group. *Right*: Effect of aluminum on inclined-plane capacity angle in spinal cord-injured rats. # $p < 0.05$ ; ## $p < 0.01$  compared with SHAM and \* $p < 0.05$ ; \*\* $p < 0.01$  compared with SCI group. All analyses were performed using the Dunnett test. Pre = preoperative; SE = standard error.

cut and stained with hematoxylin and eosin. The slides were viewed under a light microscope to study the structural changes.

#### Statistical Analysis

Data were examined using analysis of variance followed by Dunnett's multiple-range test for comparing different treatment groups. A  $p$  value of  $< 0.05$  was considered significant.

## Results

### Tarlov Score

There was no significant postoperative change in Tarlov score in sham-treated animals compared with preoperative score (Fig. 1 *left*). A highly significant decrease in Tarlov score was observed on Day 1 following SCI, which gradually recovered over a period of 10 days. Administration of aluminum significantly and dose dependently deteriorated the recovery rate following SCI.

### Sensory and Vocal Score

There was a significant decrease in the sensory/vocal score at 24 hours (Day 1) postinjury, which recovered to preinjury level on Day 5 (Fig. 1 *center*). Treatment of rats with a low dose (0.25%) of aluminum failed to produce any effect on SCI-induced deterioration of sensory/vocal score, whereas medium and high doses significantly and dose dependently delayed the recovery of sensory/vocal scores following SCI.

### Inclined-Plane Capacity Angle

Spinal compression produced a maximum deterioration of the rat's ability to walk on an inclined plane on Day 1 postinjury; this was followed by a gradual recovery over a period of 10 days. A low dose (0.25%) of aluminum failed to affect SCI-induced deterioration of capacity angle, whereas medium and high doses significantly and dose

dependently slowed the recovery of the animal's ability to walk on the inclined plane. (Fig. 1 *right*).

### Vitamin E Levels

A significant depletion of vitamin E was found in spinal cord tissue obtained in rats on Days 1 and 5 postinjury (Fig. 2). Pretreatment with aluminum produced no change in vitamin E level in noninjured animals; however, aluminum dose dependently potentiated SCI-induced depletion of vitamin E in spinal cord.

### Electrophysiological Monitoring

There was a significant reduction in the amplitude of CMEP activity following SCI at 4 hours and 24 hours, and this recovered to normal on Day 10 postinjury (Figs. 3 and 4 *left*). There was no significant difference in the amplitude of CMEP between SCI-alone group and SCI combined with aluminum-treated groups at 4 or 24 hours postinjury. However, on Day 10 the level of CMEP amplitude in aluminum and SCI-treated groups was significantly lower when compared with the SCI alone-treated group. On the other hand, SCI significantly increased the latency of the evoked potential at 4 hours and 24 hours, which partially recovered over a period of 10 days (Fig. 3b). Treatment of animals with aluminum significantly potentiated the SCI-induced increase in the latency of evoked potentials (Fig. 3b).

### Histological Examination

Spinal cord sections obtained in sham-operated animals appeared normal (Fig. 4 *upper right*). The spinal cord sections obtained from injured animals showed focal demyelination of white matter in the posterior column of spinal cord and focal loss of neurons in the gray matter. Foci of granularity and vacuolation were demonstrated in the white matter (Fig. 4 *center right*). Administration of aluminum produced more severe degenerative changes in the spinal cord following SCI (Fig. 4 *lower right*).

## Aluminum exacerbates spinal trauma

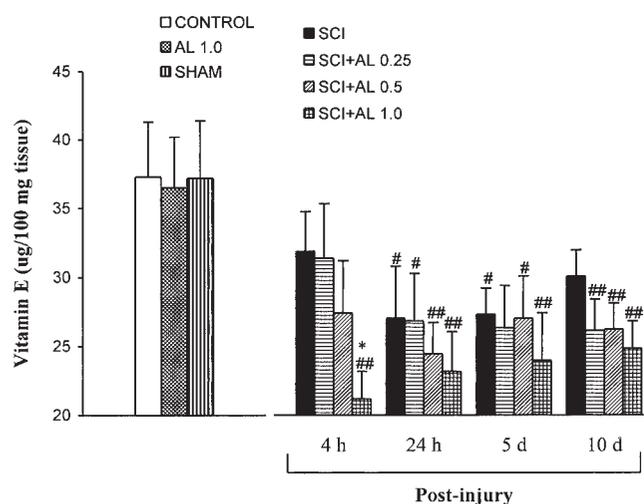


FIG. 2. Bar graph showing the effects of SCI alone and with aluminum treatment of vitamin E levels (mean  $\pm$  SE) measured in spinal cord at different postinjury periods. # $p$  < 0.05; ## $p$  < 0.01 compared with control; and \* $p$  < 0.01 compared with SCI group. Dunnett's test was used for all analyses. d = days; h = hours.

### Discussion

The results of our behavioral studies (Fig. 1) demonstrated that the compression of spinal cord for 5 minutes produced transient paraparesis in which there was a maximum motor deficit at 24 hours postinjury, which recovered gradually over a period of 10 days. These observations are in agreement with the findings of earlier investigators who observed nearly complete functional recovery following compressive SCI.<sup>34,50,68</sup> Administration of aluminum significantly and dose dependently slowed the recovery of neurobehavioral function following SCI (Fig. 1). The deleterious effects of aluminum on SCI were also confirmed by our electrophysiological (Figs. 3 and 4 left) and histopathological observations (Fig. 4 right). Aluminum can easily cross the blood-brain barrier,<sup>54</sup> and chronic exposure to this element has been shown to produce its accumulation in the brain and spinal cord, leading to neurotoxicity<sup>16,35,53</sup> and axonal degeneration.<sup>38</sup> Aluminum has been identified as an important factor underlying the development of encephalopathy that occurs in the patients receiving long-term hemodialysis.<sup>3</sup> Neurological disorders such as amyotrophic lateral sclerosis,<sup>56</sup> senile dementia of the Alzheimer type,<sup>15</sup> and parkinsonism<sup>23,56</sup> have also been associated with aluminum toxicity.

The mechanism by which aluminum impairs recovery following SCI is far from clear. Primary mechanical insult due to compression may result in a loss of axons, rupture of arterioles and venules, vascular ischemia, and disturbance of calcium homeostasis.<sup>9,78</sup> It is well established that calcium entry through voltage-sensitive channels plays an important role in the pathophysiology of secondary tissue damage following CNS trauma.<sup>84</sup> Aluminum has a high affinity for biological ligands, which typically bind essential trace elements including calcium in the body.<sup>36,74</sup> Chronic exposure of aluminum also decreases calcium-adenosine triphosphatase leading to the inhibition of calcium efflux and its accumulation in cells.<sup>74</sup> Thus, the altered calcium homeostasis and decrease in sodium/po-

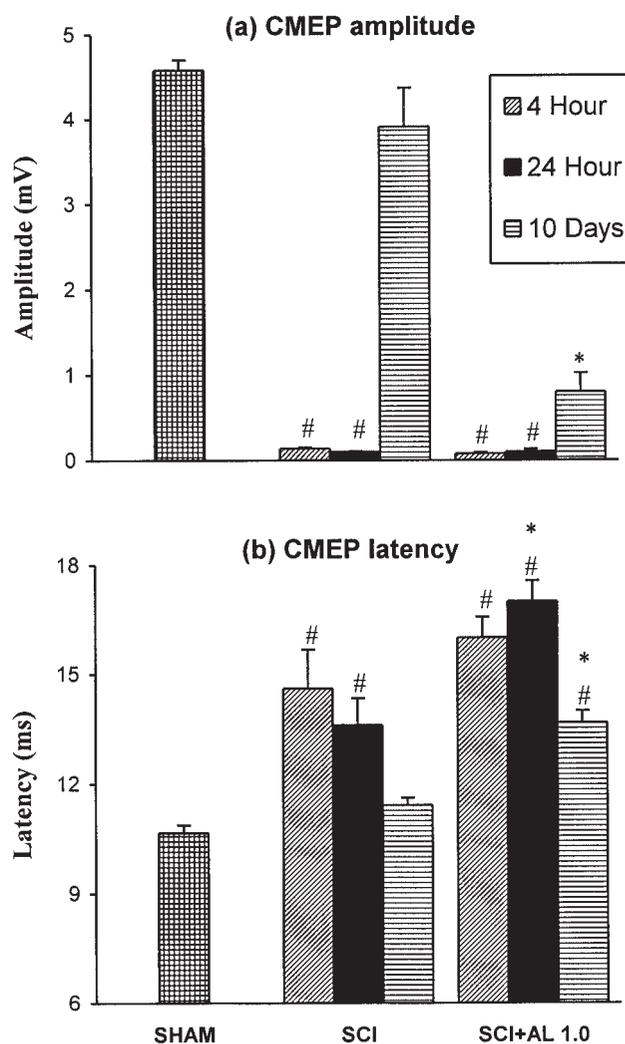


FIG. 3. Bar graphs showing amplitude (a) and latency (b) measurements of CMEP in different treatment groups at 4 hours, 24 hours, and 10 days postinjury. # $p$  < 0.01 compared with SHAM and \* $p$  < 0.01 compared with respective SCI group. Dunnett's test was used for all analyses. ms = milliseconds.

tassium-adenosine triphosphatase activity following exposure to aluminum as well as SCI may to some extent contribute to the poor neurological recovery following dual insult.

A role of monoaminergic and cholinergic transmitters in the secondary injury mechanism of CNS trauma has been suggested by several investigators.<sup>33,41,61</sup> The level of 5-hydroxytryptamine in spinal cord is rapidly elevated following blunt force or compression injury, and 5-hydroxytryptamine receptor antagonists have been shown to improve recovery following neurotraumatic injury.<sup>64</sup> Wenk and Stemmer<sup>81</sup> reported that aluminum significantly alters monoamine levels in the CNS. Furthermore, traumatic brain injury is also accompanied by activation of cholinergic activity and scopolamine, a cholinergic blocker that has been shown to attenuate motor deficits following neurotrauma.<sup>46</sup> Aluminum has been shown to enhance cholinergic activity by inhibiting cholinesterase enzyme.<sup>25</sup> Thus, the role of various transmitters as modulators of

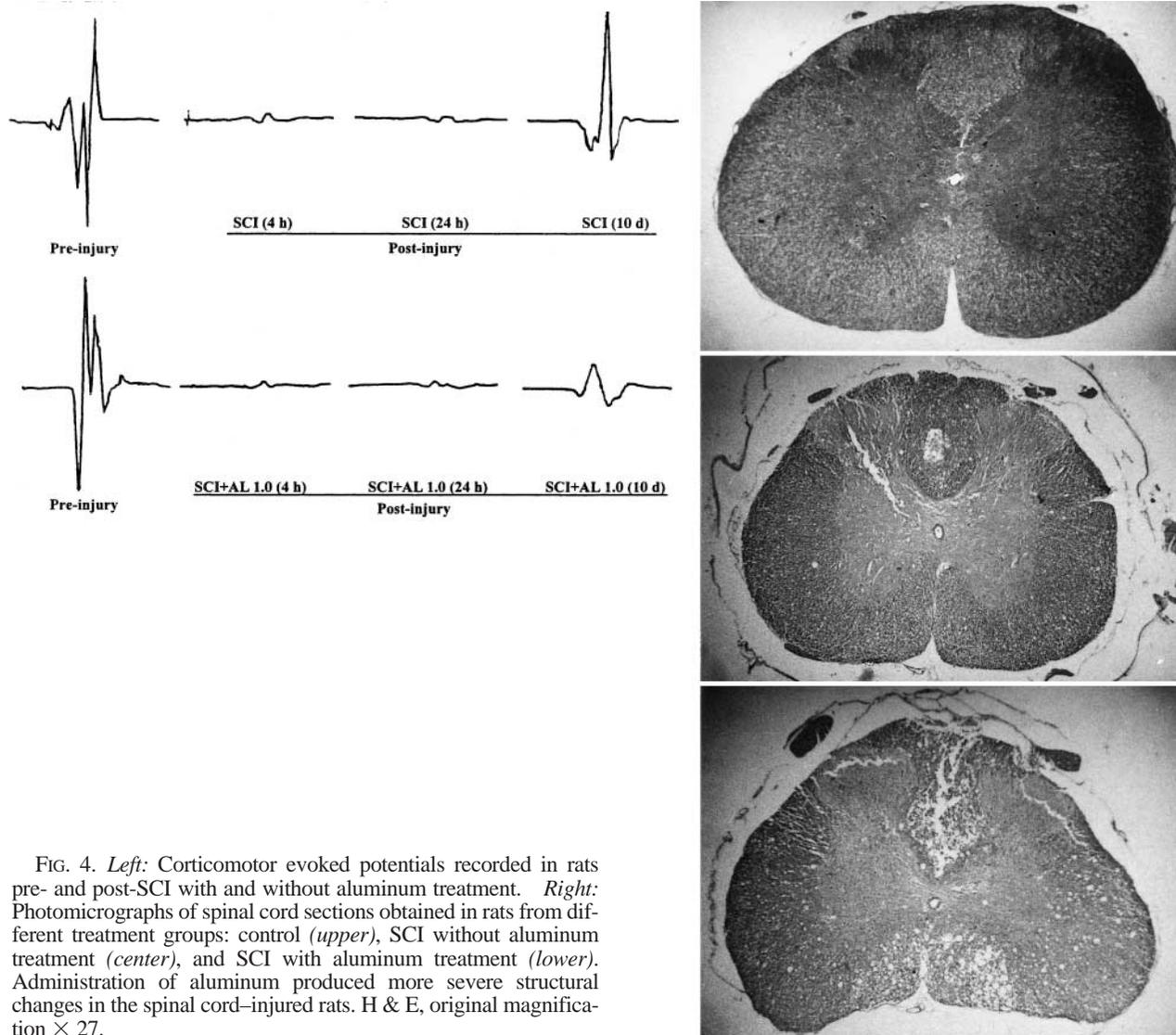


FIG. 4. *Left:* Corticomotor evoked potentials recorded in rats pre- and post-SCI with and without aluminum treatment. *Right:* Photomicrographs of spinal cord sections obtained in rats from different treatment groups: control (*upper*), SCI without aluminum treatment (*center*), and SCI with aluminum treatment (*lower*). Administration of aluminum produced more severe structural changes in the spinal cord-injured rats. H & E, original magnification  $\times 27$ .

CNS injury and their modification by aluminum warrants additional studies.

In recent studies the authors have suggested a key role of ischemia and reperfusion in neuronal damage following SCI.<sup>32,40,47</sup> The degree of early reperfusion hyperemia after decompression of spinal cord was proportional to electrophysiological recovery after neurotrauma.<sup>11</sup> The ischemia-reperfusion injury is accompanied by excessive production of oxygen-derived free radicals and enhanced lipid peroxidation,<sup>32,47,57</sup> whereas antioxidants have been shown to attenuate ischemic SCI.<sup>7,17,82</sup> Analysis of the results obtained in our biochemical studies revealed a significant depletion of vitamin E level in sections of injured spinal cord (Fig. 2). Our findings are in agreement with those reported by Saunders, et al.,<sup>63</sup> who observed a highly significant decrease in vitamin E levels in samples of injured spinal cord tissue. These authors suggested that endogenous vitamin E is quickly consumed by scavenging excessive free radicals produced as a result of a compressive injury.<sup>7,18</sup> Aluminum exposure has been shown to increase lipid peroxidation both *in vivo*<sup>12,52</sup> and *in vitro*.<sup>21,83</sup>

In our study, administration of aluminum significantly exacerbated the depletion of vitamin E in the spinal cord of injured rats (Fig. 2). Julka and Gill<sup>37</sup> also reported a significant depletion of antioxidant enzymes in different brain regions of aluminum-treated rats. Aluminum promotes physical changes in cell membranes that render them more susceptible to free radical attack (Oteiza, et al., unpublished data), suggesting its ability to aggravate oxidative stress-mediated neuronal injury following neurotrauma.

In conclusion, the results of this study clearly indicate that the neurological function and recovery rate following SCI are significantly impaired in rats exposed to aluminum. Additional studies are warranted to investigate the exact mechanism of aluminum-induced deterioration of recovery following SCI.

#### Acknowledgments

The authors thank Mrs. Rosalinda Trampe and Mrs. Anita Mabel for technical assistance and Miss Tess Jaime for typing the manuscript.

## Aluminum exacerbates spinal trauma

### References

1. Agarwal SK, Ayyash L, Gourley CS, et al: Evaluation of the developmental neuroendocrine and reproductive toxicology of aluminum. **Food Chem Toxicol** **34**:49–53, 1996
2. Al Moutaery K, Morais C, Biary N, et al: Effect of acrylamide on neurological recovery following spinal cord injury in rats. **Act Neurochir** **141**:989–999, 1999
3. Alfrey AC, LeGendre GR, Kaehny WD: The dialysis encephalopathy syndrome. Possible aluminum intoxication. **N Engl J Med** **294**:184–188, 1976
4. Allen AR: Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. **JAMA** **57**:878–880, 1911
5. Allen WM, Sansom BF: Accidental contamination of the public water supply at Lowermoor, Camelford: an assessment of the possible veterinary consequences. **Vet Rec** **124**:479–482, 1989
6. Altman P: Aluminum toxicity in dialysis patients: no evidence for a threshold serum aluminum concentration. **Nephrol Dial Transplant**:S25–S34, 1993
7. Anderson DK, Saunders RD, Demediuk P, et al: Lipid hydrolysis and peroxidation in injured spinal cord: partial protection with methylprednisolone or vitamin E and selenium. **Cent Nerv Syst Trauma** **2**:257–267, 1985
8. Bakir AA, Hryhorczuk DO, Ahmed S, et al: Hyperaluminemia in renal failure: the influence of age and citrate intake. **Clin Nephrol** **31**:40–44, 1989
9. Balentine JD, Hogan EL, Banik NL, et al: Calcium and the pathogenesis of spinal cord injury, in Dacey RG, Winn HR, Rimel RW, et al (eds): **Neurological Surgery, Trauma of the Central Nervous System**. New York: Raven Press, 1985, pp 285–295
10. Baskin DS, Simpson RK Jr: Corticomotor and somatosensory evoked potential evaluation of acute spinal cord injury in the rat. **Neurosurgery** **20**:871–877, 1987
11. Carlson GD, Minato Y, Okada A, et al: Early time-dependent decompression for spinal cord injury: vascular mechanisms of recovery. **J Neurotrauma** **14**:951–962, 1997
12. Chainy GB, Sahoo A, Swain C: Effect of aluminum on lipid peroxidation of cerebral hemisphere of chick. **Bull Environ Contam Toxicol** **50**:85–91, 1993
13. Collins WF: A review and update of experimental and clinical studies of spinal cord injury. **Paraplegia** **21**:204–219, 1983
14. Collins WF, Piepmeier J, Ogle E: The spinal cord injury problem—a review. **Cent Nerv Syst Trauma** **3**:317–331, 1986
15. Crapper DR, Krishnan SS, Dalton AJ: Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. **Science** **17**:511–513, 1973
16. Cucarella C, Montoliu C, Hermenegildo C, et al: Chronic exposure to aluminum impairs neuronal glutamate-nitric oxide-cyclic GMP pathway. **J Neurochem** **70**:1609–1614, 1998
17. Cuevas P, Carceller-Benito F, Reimers D: Administration of bovine superoxide dismutase prevents sequelae of spinal cord ischemia in the rabbit. **Anat Embryol** **179**:251–255, 1989
18. Demediuk P, Saunders RD, Anderson DK, et al: Membrane lipid changes in laminectomized and traumatized cat spinal cord. **Proc Natl Acad Sci USA** **82**:7071–7075, 1985
19. Dexter DT, Ward RJ, Wells FR, et al: Alpha-tocopherol levels in brain are not altered in Parkinson's disease. **Ann Neurol** **32**:591–593, 1992
20. Dittert DD, Warnecke G, Willert HG: Aluminum levels and stores in patients with total hip endoprostheses from TiAlV or TiAlNb alloys. **Arch Orthop Trauma Surg** **114**:133–136, 1995
21. Dominguez MC, Sole E, Goni C, et al: Effect of aluminum and lead salts on lipid peroxidation and cell survival in human skin fibroblasts. **Biol Trace Elem Res** **47**:57–67, 1995
22. Friga V, Linos A, Linos DA: Is aluminum toxicity responsible for uremic pruritis in chronic hemodialysis patients? **Nephron** **75**:48–53, 1997
23. Garruto RM, Fukastu R, Yanagihara R, et al: Imaging of calcium and aluminum in neurofibrillary tangle-bearing neurons in parkinsonism-dementia of Guam. **Proc Natl Acad Sci USA** **81**:1875–1879, 1984
24. Giordano R, Costantini S: Some aspects related to the presence of aluminum in waters. **Ann Ist Super Sanita** **29**:305–311, 1993
25. Golub MS, Gershwin ME, Donald JM, et al: Maternal and developmental toxicity of chronic aluminum exposure in mice. **Fundam Appl Toxicol** **8**:346–357, 1987
26. Gomez M, Sanchez DJ, Llobet JM, et al: The effect of age on aluminum retention in rats. **Toxicology** **116**:1–8, 1997
27. Greger JL, Bula EN, Gum ET: Mineral metabolism of rats fed moderate levels of various aluminum compounds for short periods of time. **J Nutr** **115**:1708–1716, 1985
28. Grissom TE, Mitzel HC, Bunegin L, et al: The effect of anesthetics on neurologic outcome during the recovery period of spinal cord injury in rats. **Anesth Analg** **79**:66–74, 1994
29. Hall ED, Wolf DL: A pharmacological analysis of the pathophysiological mechanisms of posttraumatic spinal cord ischemia. **J Neurosurg** **64**:951–961, 1986
30. Hall ED, Yonkers PA, Horan KL: Correlation between attenuation of posttraumatic spinal cord ischemia and preservation of tissue vitamin E by the 21-aminosteroid U74006 F: evidence for an in vivo antioxidant mechanism. **J Neurotrauma** **6**:169–176, 1989
31. Halt PS, Swanson RA, Faden AI: Alcohol exacerbates behavioral and neurochemical effects of rat spinal cord trauma. **Arch Neurol** **49**:1178–1184, 1992
32. Hamada Y, Ikata T, Katoh S, et al: Roles of nitric oxide in compression injury of rat spinal cord. **Free Rad Biol Med** **20**:1–9, 1996
33. Hashimoto T, Fukuda N: Contribution of serotonin neurons to the functional recovery after spinal cord injury in rats. **Brain Res** **539**:263–270, 1991
34. Holtz A, Nystrom B, Gerdin B: Relation between spinal cord blood flow and functional recovery after blocking weight-induced spinal cord injury in rats. **Neurosurgery** **26**:952–957, 1990
35. Jope RS, Johnson GV: Neurotoxic effects of dietary aluminum. **Ciba Found Symp** **169**:24–262, 1992
36. Julka D, Gill KD: Altered calcium homeostasis: a possible mechanism of aluminium-induced neurotoxicity. **Biochim Biophys Acta** **1315**:47–54, 1996
37. Julka D, Gill KD: Effect of aluminum on regional brain antioxidant defense status in Wistar rats. **Res Exp Med** **196**:187–194, 1996
38. Kihira T, Yoshida S, Komoto J, et al: Aluminum-induced model of motor neuron degeneration: subperineurial injection of aluminum in rabbits. **Neurotoxicology** **16**:413–424, 1995
39. Koo WW, Kaplan LA: Aluminum and bone disorders: with specific reference to aluminum contamination of infant nutrients. **J Am Coll Nutr** **7**:199–214, 1988
40. Kraus KH: The pathophysiology of spinal cord injury and its clinical implications. **Semin Vet Med Surg (Small Anim)** **11**:201–207, 1996
41. Kurihara M: Role of monoamines in experimental spinal cord injury in rats. Relationship between Na<sup>+</sup>-K<sup>+</sup>-ATPase and lipid peroxidation. **J Neurosurg** **62**:743–749, 1985
42. Lin JL, Yang YJ, Yang SS, et al: Aluminum utensils contribute to aluminum accumulation in patients with renal disease. **Am J Kidney Dis** **30**:653–658, 1997
43. Lione A: The prophylactic reduction of aluminium intake. **Food Chem Toxicol** **21**:103–109, 1983
44. Little JW, Harris RM, Lerner SJ: Immobilization impairs recovery after spinal cord injury. **Arch Phys Med Rehabil** **72**:408–412, 1991
45. Lopez FF, Cabrera C, Lorenzo ML, et al: Aluminium levels in wine, beer and other alcoholic beverages consumed in Spain. **Sci Total Environ** **220**:1–9, 1998
46. Lyeth BG, Dixon CE, Hamm RJ, et al: Effects of anticholinergic treatment on transient behavioral suppression and physio-

- logical responses following concussive brain injury to the rat. **Brain Res** **448**:88–97, 1988
47. Mauney MC, Blackbourne LH, Langenburg SE, et al: Prevention of spinal cord injury after repair of the thoracic or thoracoabdominal aorta. **Ann Thorac Surg** **59**:245–252, 1995
  48. Mayor GH, Keiser JA, Makdani D, et al: Aluminum absorption and distribution: effect of parathyroid hormone. **Science** **197**:1187–1189, 1977
  49. Nagy E, Jobst K: The kinetics of aluminium-containing antacid absorption in man. **Eur J Clin Chem Clin Biochem** **32**:119–121, 1994
  50. Nystrom B, Berglund JE, Bergquist E: Methodological analysis of an experimental spinal cord compression model in the rat. **Acta Neurol Scand** **78**:460–466, 1988
  51. Nystrom B, Berglund JE: Spinal cord restitution following compression injuries in rats. **Acta Neurol Scand** **78**:467–472, 1988
  52. Ohtawa M, Seko M, Takayama F: Effect of aluminum ingestion on lipid peroxidation in rats. **Chem Pharm Bull** **31**:1415–1418, 1983
  53. Oteiza PI, Keen CL, Han B, et al: Aluminum accumulation and neurotoxicity in Swiss-Webster mice after long-term dietary exposure to aluminum and citrate. **Metabolism** **42**:1296–1300, 1993
  54. Peng JH, Xu ZC, Xu ZX, et al: Aluminum-induced acute cholinergic neurotoxicity in rat. **Mol Chem Neuropathol** **17**:79–89, 1992
  55. Perez-Espejo MA, Haghghi SS, Adelstein EH, et al: The effects of taxol, methylprednisolone, and 4-aminopyridine in compressive spinal cord injury: a qualitative experimental study. **Surg Neurol** **46**:350–357, 1996
  56. Perl DP, Gajdusek DC, Garruto RM, et al: Intraneuronal aluminum accumulation in amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam. **Science** **217**:1053–1055, 1982
  57. Qayumi AK, Janusz MT, Jamieson WR, et al: Pharmacologic interventions for prevention of spinal cord injury caused by aortic crossclamping. **J Thorac Cardiovasc Surg** **104**:256–261, 1992
  58. Rauch H, Fleischer F, Bohrer H, et al: Serum aluminium levels of intensive care patients treated with two different antacids for prevention of stress ulceration. **Intensive Care Med** **15**:84–86, 1989
  59. Rivlin AS, Tator CH: Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. **Surg Neurol** **10**:39–43, 1978
  60. Rivlin AS, Tator CH: Objective clinical assessment of motor function after experimental spinal cord injury in the rat. **J Neurosurg** **47**:577–581, 1977
  61. Salzman SK, Hirofuji E, Lladós-Eckman C, et al: Monoaminergic responses to spinal trauma. Participation of serotonin in posttraumatic progression of neural damage. **J Neurosurg** **66**:431–439, 1987
  62. Sandler AN, Tator CH: Review of the effect of spinal cord trauma on the vessels and blood flow in the spinal cord. **J Neurosurg** **45**:638–646, 1976
  63. Saunders RD, Dugan LL, Demediuk P, et al: Effects of methylprednisolone and the combination of alpha-tocopherol and selenium on arachidonic acid metabolism and lipid peroxidation in traumatized spinal cord tissue. **J Neurochem** **49**:24–31, 1987
  64. Sharma HS, Olsson Y: Edema formation and cellular alteration following spinal cord injury in the rat and their modification with p-chlorophenylalanine. **Acta Neuropathol** **79**:604–610, 1990
  65. Simpson RK, Hsu CY, Dimitrijevic MR: The experimental basis for early pharmacological intervention in spinal cord injury. **Paraplegia** **29**:364–372, 1991
  66. Smith AJ, Faugere MC, Abreo K, et al: Aluminum-related bone disease in mild and advanced renal failure: evidence for high prevalence and morbidity and studies on etiology and diagnosis. **Am J Nephrol** **6**:275–283, 1986
  67. Taoka Y, Okajima K, Uchiba M, et al: Reduction of spinal cord injury by administration of iloprost, a stable prostacyclin analog. **J Neurosurg** **86**:1007–1011, 1997
  68. Tariq M, Morais C, Kishore PN, et al: Neurological recovery in diabetic rats following spinal cord injury. **J Neurotrauma** **15**:239–251, 1998
  69. Tariq M, Morais C, Sujata B, et al: Aluminum exacerbates cyclosporin induced nephrotoxicity in rats. **Ren Fail** **21**:35–48, 1999
  70. Tarlov IM, Klinger H: Spinal cord compression studies. **Ann Arch Neurol Psychiatry** **71**:275–290, 1954
  71. Tarlov IM, Klinger H, Vitale S: Spinal cord compression studies. I. Experimental techniques to produce acute and gradual compression. **Arch Neurol Psychiatry** **70**:813–819, 1953
  72. Tator CH, Edmonds VE: Acute spinal cord injury: analysis of epidemiological factors. **Can J Surg** **22**:575–578, 1979
  73. Tator CH, Fehlings MG: Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. **J Neurosurg** **75**:15–26, 1991
  74. Trapp GA: Interaction of aluminum with cofactors, enzymes, and other proteins. **Kidney Int Suppl** **18**:S12–S16, 1986
  75. Verbeeck RM, Driessens FC, Rotjans J: Aluminium in tooth pastes and Alzheimer's disease. **Acta Stomatol Belg** **87**:141–144, 1990
  76. von Bonsdorff M, Sipila R, Pitkanen E: Correction of haemodialysis-associated anaemia by deferoxamine. Effects on serum aluminum and iron overload. **Scand J Urol Nephrol Suppl** **131**:49–54, 1990
  77. von Euler M, Li-Li M, Whittemore S, et al: No protective effect of the NMDA antagonist memantine in experimental spinal cord injuries. **J Neurotrauma** **14**:53–61, 1997
  78. Wallace CM, Tator CH, Frazer P: Relationship between post-traumatic ischemia and hemorrhage in the injured rat spinal cord as shown by colloidal carbon angiography. **Neurosurgery** **18**:433–439, 1986
  79. Walton J, Tuniz C, Fink D, et al: Uptake of trace amounts of aluminum into the brain from drinking water. **Neurotoxicology** **16**:187–190, 1995
  80. Wang CX, Nuttin BJ, Gybels JM: Motor evoked potentials in a static load model of spinal cord injury in the rat. **Neurosurgery** **32**:269–273, 1993
  81. Wenk GL, Stemmer KL: Activity of the enzymes dopamine- $\beta$ -hydroxylase and phenylethanolamine-N-methyltransferase in discrete brain regions of the copper-zinc deficient rat following aluminum ingestion. **Neurotoxicology** **3**:93–99, 1982
  82. Wisselink W, Money SR, Crockett DE, et al: Ischemia-reperfusion injury of the spinal cord: protective effect of the hydroxyl radical scavenger dimethylthiourea. **J Vasc Surg** **20**:444–450, 1994
  83. Xie CX, Yokel RA: Aluminum facilitation of iron-mediated lipid peroxidation is dependent on substrate, pH and aluminum and iron concentrations. **Arch Biochem Biophys** **327**:222–226, 1996
  84. Young W: The role of calcium in spinal cord injury. **Cent Nerv Syst Trauma** **2**:109–114, 1985

Manuscript received August 10, 1999.

Accepted in final form June 20, 2000.

The authors received financial assistance from the Research and Ethics Committee of Riyadh Armed Forces Hospital.

Address reprint requests to: Mohammad Tariq, Ph.D., F.R.C.Path., F.R.C.S., Research Center, Armed Forces Hospital, P.O. Box 7897 (W-912). Riyadh 11159, Saudi Arabia.