Eklem Hastalıkları ve Cerrahisi
Joint Diseases and Related Surgery

Experimental Study / *Deneysel Çalışma*

Eklem Hastalık Cerrahisi
2010;21(2):110-115

Evaluation of urinary bladder function after acute spinal cord injury: an experimental study

Mesane kası fonksiyonunun akut spinal kord yaralanması sonrası değerlendirilmesi: Deneysel çalışma

Zekeriya Öztemür, M.D.,[1] Gündüz Tezeren, M.D.,[1] İhsan Bağcivan, M.D.,[2] Bülent Saraç, M.D.,[2] Nedim Durmuş, M.D.,[2] Reyhan Eğilmez, M.D.[3]

Departments of [1]Orthopedics and Traumatology, [2]Pharmacology and [3]Pathology, Medicine Faculty of Cumhuriyet University, Sivas, Turkey

Objectives: The aim of this study was to evaluate the efficacy of early surgical decompression of acute spinal cord injury through the evaluation of urinary bladder function in rabbits.

Materials and methods: The study was done with 21 New Zealand male rabbits which were 9 to 12 months in age, and weighed an average of 2438 grams (range 2150 to 3550 g). The animals were assigned into four groups as follows: a control group (n=5), a laminectomy group (n=6), a 15-second compression group (n=5) and a 60-second compression group (n=5). A 60 gram compression force was applied on both compression groups with aneurysm clips. All rabbits were sacrificed seven days postoperatively. Urinary bladder tissues were dissected and in vitro relaxation and contraction tests were performed in organ baths.

Results: At the beginning of each experiment, 80 mM KCl was added to the isolated organ bath with no significant difference among all four groups ($p>0.05$). Carbachol was then added to the organ bath and contraction responses were obtained. Carbachol contraction responses were calculated as the percentage of the 80 mM KCl contraction responses, with compression groups showing significant difference from control and sham-operated groups ($p<0.05$). Electrical field stimulation responses were obtained for all group preparations at 4, 8, 16, 32 Hz frequencies, and showed significant difference in the 15 and 60-second compression groups ($p<0.05$). The contractility was assessed using E-max and pD2 values. All groups exhibited same pD2 values.

Conclusion: The study demonstrated a slightly better outcome for bladder contractility with early decompression. However, there was no significant difference between early and delayed decompression groups.

Key words: Early decompression; spinal cord injury; spine; surgery; urinary bladder.

Amaç: Bu çalışmada tavşan mesanesi kullanarak akut spinal kord yaralanmasında erken cerrahi dekompresyonun yararları değerlendirildi.

Gereç ve yöntemler: Bu çalışma, 9-12 aylık ve ortalama ağırlıkları 2438 gr (dağılım; 2150-3550 gr) olan 21 erkek Yeni Zelanda tavşanı kullanılarak yapıldı. Hayvanlar kontrol grubu (n=5), laminektomi grubu (n=6), 15-saniyelik kompresyon grubu (n=5) ve 60-saniyelik kompresyon grubu (n=5) olmak üzere dört gruba ayrıldı. Her iki kompresyon grubuna da bir anevrizma klipsiyle 60 gramlık kompresyon gücü uygulandı. Bütün tavşanlar cerrahi işlemden sonraki yedinci günde sakrifiye edildi. Mesane dokusu izole edildi ve gevşeme-kasılma yanıtları alınmak üzere organ banyosuna asıldı.

Bulgular: Bütün deneylerden önce izole organ banyosuna 80 mM KCl eklendi ve dört grup arasında anlamlı bir fark bulunmadı ($p>0.05$). Sonra ortama karbakol eklenerek kasılma yanıtları alındı. Karbakol kasılma yanıtları 80 mM KCl yanıtlarının yüzdesi olarak hesaplandı ve kompresyon gruplarında kontrol ve sham cerrahi gruplarına göre anlamlı farklılıklar görüldü ($p<0.05$). Bütün gruplar için elektriksel alan uyarısı 4, 8, 16, 32 Hz frekanslarında uygulandı, 15 ve 60-saniyelik kompresyon gruplarında anlamlı farklılık bulundu ($p<0.05$). Kasılma değerleri E-max ve pD2 değerleri kullanılarak ifade edildi ve tüm grupların pD2 değerleri aynı bulundu.

Sonuç: Sonuç olarak, bu çalışmada erken cerrahi dekompresyonun, mesane kontraktilitesini biraz daha iyi koruduğu gösterildi. Ancak erken ve gecikmiş dekompresyon grupları arasındaki farkın istatistiksel olarak anlamlı olmadığı bulundu.

Anahtar sözcükler: Erken dekompresyon; spinal kord yaralanması; omurilik; cerrahi; mesane.

- *Received: September 22, 2008 Accepted: July 31, 2009*
- Correspondence: Zekeriya Öztemür, M.D. Cumhuriyet Üniversitesi Tıp Fakültesi Ortopedi ve Travmatoloji Anabilim Dalı, 58140 Sivas, Turkey. Tel: +90 346 - 258 06 37 Fax: +90 346 - 258 13 04 e-mail: oztemur@cumhuriyet.edu.tr
- *Cumhuriyet Üniversitesi Bilimsel Araştırma Proje Birimi (CUBAP) tarafından T-237 nolu Tıpta Uzmanlık Tez Projesi olarak desteklenmiştir.*

Traumatic spinal cord injury (SCI) results in the loss of spinal cord tissue and permanent neurological deficits. In 50% of patients, injury is initially incomplete; some residual function remains.[1,2] Vertebral fracture or dislocation is a frequent cause of spinal cord bruising or contusion.[1,3]

Following SCI, the normal control of voiding is lost due to the interruption of the spino-bulbo-spinal micturition reflex pathway, which results in a series of pathophysiological and morphological changes in both the spinal cord and the bladder.[4-6] In the lower urinary tract, the coordinated function of the bladder and sphincter ceases. Bladder-sphincter dyssynergia, a functional obstruction of the bladder outlet, develops and results in increased bladder mass, and increased expression of neurotrophic factors.[4,7-9]

Bladder dysfunction has consistently been ranked as one of the top concerns among paraplegics and quadriplegics, usually of higher importance than the loss of locomotion.[10-12]

Most studies examining lower urinary traction function after SCI have used completely transected animals. Initially after thoracic spinal cord transaction, the bladder is areflexic, causing urinary retention and bladder enlargement. Hyperreflexic bladder contractions re-appear after 7-10 days; voiding is possible but inefficient.[10,13] A few studies have used partial compression or acute compression models such as the weight drop model.[1,14] We performed SCI with the clip compression model.[15] The aim of this study was to evaluate the efficacy of early surgical decompression of acute SCI in the experimental animal model by evaluating the alteration of cholinergic system efficacy on smooth muscle of the bladder.

MATERIALS AND METHODS

Animals

Twenty-one male New Zealand rabbits weighing an average of 2438 grams (range; 2150-3550 g) and aged 9-12 months were used. Food and water were provided appropriately and the animals were housed in the cages at a temperature of 20±2 ºC. The approval for this study was received from the Animal Research Ethics Committee of Cumhuriyet University.

Spinal cord compression model

The animals were anesthetized with a mixture of 3 mg/kg xylazin hydrochloride (Rompun, Bayer AG, Germany) and 10 mg/kg ketamine hydrochloride (Ketalar, Pfizer Inc, USA). After the animals were placed in the prone position with pillows, the surgical site was shaved and scrubbed with a povidone-iodine solution. 100 mg/kg ceftriaxone was injected intramuscularly (Nevakson, Mustafa Nevzat AŞ, Turkey) as an antibiotic prophylaxis, a midline skin incision over the spinous process about 5 cm length was performed under aseptic conditions. A laminectomy was then carried out using a small Kerrison rongeur at T12 vertebra exposing the dural sac. Epidural bleeding was controlled with the gel-foam. Following compression-decompression procedures, the wound was irrigated with sterile saline, then, the fascia was closed with 3-0 vicryl sutures, and the skin was closed with 4-0 prolene sutures. For analgesia, 4 mg/kg carprofen (Rimadyl, Pfizer Inc., USA) was administered to the operated animals once a day. Optimum care was taken to avoid pressure ulcers and the animals were forced to urinate through abdomen massages and by using hot packs. Seven days after compression-decompression of the spinal cord, the animals were sacrificed with 200 mg/kg Thiopentane sodium intravenous (i.v) (Pental Sodium, İE Ulagay Türk AŞ, Turkey).

The animals were divided into four groups; group A, which included five animals, was the control group, group B, which included six animals, was a sham-operated (laminectomy) group, group C included five animals and was the 15 second compression group, and group D had five animals for the 60 second compression group. The control group had no operation, while the animals in sham-operated group underwent laminectomy procedures as described above. The remaining ten (15 second compression group and 60 second compression group) animals underwent Rivlin and Tator[15] extradural "clip compression injury" procedures for inducing SCI. For this purpose, a 60 gram closing force aneurysm clip (Sugita Aneurysm Clips, Type Temporary Mini, Mizuho Ikakogyo Co. Ltd., Japan) was used. The animals in group C sustained 15 seconds of spinal cord compression, then decompression was performed by removing the clips. The remaining five animals (group D- 60 second compression group) was subjected to 60 seconds of spinal cord compression followed by decompression in the same manner.

Histopathological evaluation

The spinal cords were removed immediately after sacrifice, and divided into three parts: an injured site, and the parts proximal and distal to the injured site. The segments were immersed in 10% formaldehyde solution and each segment was prepared into slices. The slices were embedded in paraffin and stained with hematoxylin and eosin.

Isometric measurements

Laparatomy was performed after the rabbits were anesthetized with intravenous sodium pentobarbital (50 mg/kg). The bladder was removed, via clamping and cutting of the cystic duct, and placed in a Krebs-bicarbonate solution (KBS). The rabbit was then killed with an additional intravenous bolus of sodium pentobarbital. The bladder was opened with a longitudinal incision and full thickness muscle strips (2x10 mm) were cut along the longitudinal axis.[16] The strips were maintained in a 10 mL organ bath containing KBS solution with the following composition: 120 mM NaCl, 4.6 mM KCl, 2.5 mM $CaCl_2$, 22 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, and 11.5 mM glucose.

The solution was gassed with 95% O_2 and 5% CO_2 during the study and temperature was maintained at 37 °C by a thermoregulated water circuit. One end of each preparation was attached to the bottom of the organ bath, while the other end was tied to a force transducer (Grass FT 03; Grass Instruments, Quincy, MA, USA) connected to pen polygraph (Grass 79 E). After mounting, each strip was allowed to equilibrate with a basal tension of 1.0 g for one hour. The Krebs-bicarbonate solution was replaced with fresh solution every 15 minutes during this time period. Bladder strips from all four groups were exposed to 80 mM KCl before exposure to drugs. All strips were allowed to equilibrate for 30 minutes and were then contracted with carbachol (Sigma Chemicals Co., St. Louis, MO, USA) in stepwise cumulative doses. The concentration of carbachol (10^{-4}-10^{-8} M) in the bathing medium was increased only after the response to the previous concentration had attained a maximal and steady level. Carbachol contraction responses were calculated as the percentage of 80 mM KCl contraction responses.

Electrical field stimulation (EFS)

Stimulation of gallbladder muscle strips was provided by two parallel platinum electrodes. Sequential frequencies of 1-64 Hz, as square-wave pulses of 60 V (1 ms), were delivered at 10 second intervals by a current amplifier and a stimulator (S88; Grass Instruments). The strips were allowed to return to their baseline tension between the tests. Frequency response contractions of bladder muscle strips at 1-64 Hz were obtained. The EFS-induced contractile responses were calculated as the percentage of 80 mM KCl contraction responses.

Solution and Chemicals used

Carbachol was purchased from Sigma. All drugs were dissolved in the bidistilled water and freshly prepared on the day of the experiment.

Statistical analysis

Data values are expressed as means ± standard error of the mean (SEM) of the results obtained from muscle strips. ANOVA and Scheffé F testing were used to compare groups. P values of less than 0.05 were considered to indicate statistical significance. The statistical analysis was performed with SPSS for Windows version 10.0 statistical software (SPSS, Inc., Chicago, Illinois, USA).

RESULTS

At the beginning of each experiment, 80 mM KCl was added to the isolated organ bath and there was no significant difference among all four groups ($p>0.05$; Figure 1). Carbachol was then added to organ bath and contraction responses were obtained. For the 15 and 60 second compression groups, carbachol contraction responses were calculated as the percentage of 80 mM KCl contraction responses. There was a significant dif-

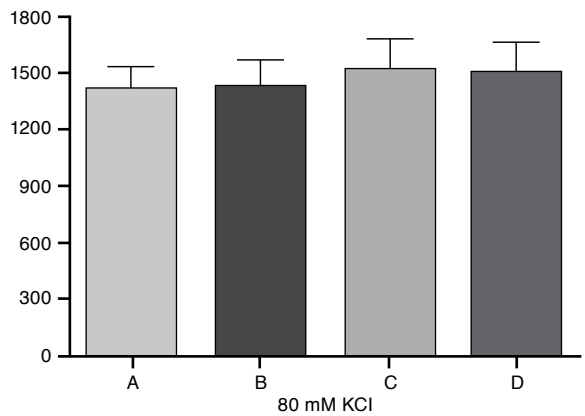

Figure 1. (A) Contraction responses of control, (B) sham-operated, (C) 15 seconds compression and (D) 60 seconds compression groups with 80 mM KCl in miligrams.

ference between the control and sham-operated groups ($p<0.05$; Figure 2). Electrical field stimulation responses were obtained for all group preparations at 4, 8, 16, 32 Hz frequencies and there was a significant difference for the 15 and 60 second compression groups ($p<0.05$; Figure 3). The contractility was assessed using Emax and pD2 values (Table I). All groups exhibited same pD2 values.

The spinal cord slides were examined by light microscopy. In the 15 second compression group, eosinophilia, vacuolization, perivascular halos, chromatolysis and hyperemia on the proximal end of the lesion were seen. There were diffuse hemorrhages, hyperemia, vacuolization, perivascular halo, chromatolysis on the lesion site while gliosis, eosinophilia, perivascular halo, chromatolysis on the distal end of the lesion were observed. In 60-second group, diffuse nucleus loss, hyperemia, vacuolization, eosinophilia, chromatolysis on the proximal end of the lesion were observed, while hemorrhages, hyperemia, eosinophilia, chromatolysis on the lesion site and hyperemia, eosinophilia, and chromatolysis on the distal end of the lesion were noted.

DISCUSSION

The goal of the treatment of spinal cord injury is to preserve residual neurologic function, avoid secondary injury, and restore spinal alignment and stability. While future treatment may include neurotrophic factor infusion, and neural tissue transplantation, it should be focused on preventing secondary injury and allowing natural recovery of the injured spinal cord.[17]

The timing of surgery for spinal cord injuries is controversial. In patients with incomplete spinal cord injuries, some authors state that there is no difference between early and delayed decompression,[18-20] whereas others favor early surgical stabilization.[18-21] Even if decompression previously had a poor reputation, the debate has recently shifted to what the timing for decompression should be. Fehlings et al.[22] suggested that analysis of the literature did not allow definite conclusions to be drawn regarding appropriate timing of intervention and Leventhal[23] suggested that there was no conclusive evidence that early surgical decompression and stabilization improved neurological recovery or that neurological recovery was compromised by a delay of several days. On the other hand, Clohisy et al.[24] found that early anterior decompression for traumatic injuries at the thoracolumbar junction was associated with improved rates of neurologic recovery when compared to delayed decompression. Duh et al.[25] concluded that their study did not provide clinically relevant evidence concerning the efficacy of timing or the value of surgery in treating patients with spinal cord injuries. The same observation was mentioned by Tator et al.[26]

In the reviewed literature, various animal species were used for these studies as well as various

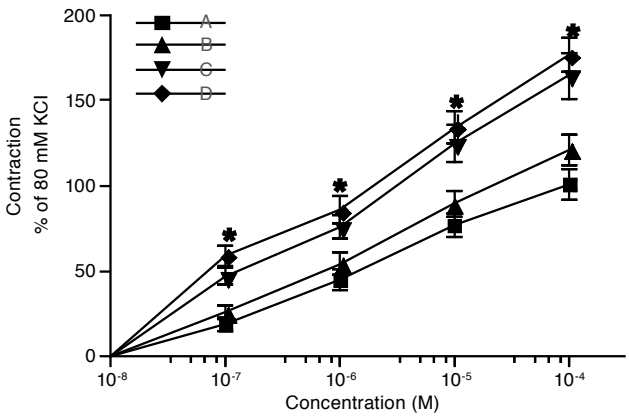

Figure 2. (A) Contraction responses obtained with cumulative concentration of carbachol from control, (B) sham-operated, (C) 15 seconds compression and (D) 60 seconds compression groups as % of 80 mM KCl. Data are expressed as the means ± SEM. *: $P<0.05$ denotes significant difference from control (A), sham-operated (B) and 60 seconds (D) compression groups.

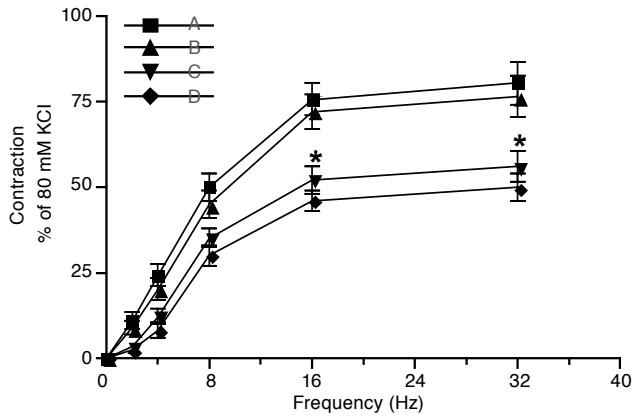

Figure 3. (A) Contraction responses obtained with EFS from control, (B) sham-operated, (C) 15 seconds compression and (D) 60 seconds compression groups as % of 80 mM KCl. Data are expressed as the means ± SEM. *: $P<0.05$ denotes significant difference from control (A), sham-operated (B) and 60 (D) seconds compression groups.

injury models. The models include extradural balloon compression of the spinal cord[27] a weight drop technique,[26] a technique in which a weight compresses the cord by a device, similar to a technique by Black et al.[28] constricting the cord with a band[29] and extradural clip compression injury by Rivlin and Tator[15] We used the latter model for being easy, reproducible and also mimicking a clinical situation in which a compression is caused by a bony fragment.

Bladder dysfunction is a serious complication of SCI. Thoracolumbar cord lesions above the conus medullaris result in a hyperreflexic bladder, while conus medullaris injuries which occur between T11 and L2 or cauda equina injuries result in flaccid paralysis.[27] Mure et al.[29] examined the relationship between measurements of neurological recovery and controlled voiding by using cluster analysis and found that early recovery of controlled voiding was predictive of motor recovery. In the study of Beric and Light,[30] patients with an early suprasacral spinal cord injury underwent comprehensive neurourological evaluation to determine if there was any correlation between the return of detrusor function and neural function of the sacral cord: they found that the patients with normal lumbosacral evoked potentials predicted return of bladder function.

In this study, there was no difference between KCL contraction responses in the four groups. Carbachol contraction responses increased in 15 and 60 second compression groups, whereas EFS contraction responses decreased in 15 and 60 second compression groups. KCL is a substance which produces contractions without a receptor mediator; no change in KCL contractions in four groups show that contraction functions of bladder muscles have not deteriorated. The increase of carbachol mediator receptor contraction responses both compression groups indicates the possible increase of amount and/or activity of muscarinic receptors in bladder muscle. The decrease of EFS contraction responses in the compression groups may indicate a decrease of Ach release from cholinergic nerves. On the other hand, increase of carbachol responses may be due to up-regulation of Ach receptors after the decrease of Ach.

In conclusion, the present study demonstrates that early decompression appears to manifest slightly better outcome in bladder contractility. However, there is no statistically significant difference between early and delayed decompression groups. In addition, according to histopathological assessment of the spinal cord, early and delayed decompression appear have a similar outcome.

REFERENCES

1. Teng YD, Mocchetti I, Taveira-DaSilva AM, Gillis RA, Wrathall JR. Basic fibroblast growth factor increases long-term survival of spinal motor neurons and improves respiratory function after experimental spinal cord injury. J Neurosci 1999;19:7037-47.
2. Bracken MB, Shepard MJ, Collins WF, Holford TR, Young W, Baskin DS, et al. A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. N Engl J Med 1990;322:1405-11.
3. Kurtzke JF. Epidemiology of spinal cord injury. Neurol Neurocir Psiquiatr 1977;18(2-3 Suppl):157-91.
4. Somogyi GT, Zernova GV, Yoshiyama M, Rocha JN, Smith CP, de Groat WC. Change in muscarinic modulation of transmitter release in the rat urinary bladder after spinal cord injury. Neurochem Int 2003;43:73-7.
5. de Groat WC. Mechanisms underlying the recovery of lower urinary tract function following spinal cord injury. Paraplegia 1995;33:493-505.
6. de Groat WC, Booth AM, Yoshimura N. Neurophysiology of micturition and its modification in animal models of human disease. In: Maggi CA, editor. The autonomic nervous system. Nervous control of the urogenital system. Vol. 3. 1st ed. London: Harwood Academic Publishers; 1993. p. 227-90.
7. Steers WD, Kolbeck S, Creedon D, Tuttle JB. Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. J Clin Invest 1991;88:1709-15.
8. Kruse MN, Bray LA, de Groat WC. Influence of spinal cord injury on the morphology of bladder afferent and efferent neurons. J Auton Nerv Syst 1995;54:215-24.
9. Vizzard MA. Changes in urinary bladder neurotrophic factor mRNA and NGF protein following urinary bladder dysfunction. Exp Neurol 2000;161:273-84.
10. Leung PY, Johnson CS, Wrathall JR. Comparison of the effects of complete and incomplete spinal cord injury on lower urinary tract function as evaluated in unanesthetized rats. Exp Neurol 2007;208:80-91.
11. Anderson KD. Targeting recovery: priorities of the spinal cord-injured population. J Neurotrauma 2004; 21:1371-83.
12. Benevento BT, Sipski ML. Neurogenic bladder, neurogenic bowel, and sexual dysfunction in people with spinal cord injury. Phys Ther 2002;82:601-12.
13. Chancellor MB, Rivas DA, Huang B, Kelly G, Salzman SK. Micturition patterns after spinal trauma as a measure of autonomic functional recovery. J Urol 1994; 151:250-4.

14. Allen AR. Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. JAMA 1911;57:878-80.
15. Rivlin AS, Tator CH. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg Neurol 1978;10:38-43.
16. Yildirim S, Aydin C, Koyuncu A, Bagcivan I, Sarac B, Sarioglu Y. Enhancement of EFS-induced contractions, by agmatine, in guinea pig gallbladder smooth muscle strips. J Gastroenterol 2005;40:498-503.
17. Lee TT, Green BA. Advances in the management of acute spinal cord injury. Orthop Clin North Am 2002; 33:311-5.
18. Carlson GD, Minato Y, Okada A, Gorden CD, Warden KE, Barbeau JM, et al. Early time-dependent decompression for spinal cord injury: vascular mechanisms of recovery. J Neurotrauma 1997;14:951-62.
19. Chapman JR, Anderson PA. Thoracolumbar spine fractures with neurologic deficit. Orthop Clin North Am 1994;25:595-612.
20. Jarmundowicz W, Lawicki B, Orkisz S. The effect of prolonged spinal cord compression on the extent of morphological changes in experimental spinal cord injury in rabbits. Neurol Neurochir Pol 1997;31:1177-88. [Abstract]
21. Jarmundowicz W, Tosik D, Chlebinski J, Górkiewicz Z. The effect of early decompression on the extent of changes in spinal cord microcirculation in experimental traumatic injury to the cord in rabbits. Neurol Neurochir Pol 1997;31:1167-75. [Abstract]
22. Fehlings MG, Sekhon LH, Tator C. The role and timing of decompression in acute spinal cord injury: what do we know? What should we do? Spine (Phila Pa 1976) 2001;26(24 Suppl):S101-10.
23. Leventhal MR. Fractures, dislocations and fracture-dislocations of spine. In: Crenshaw AH, editor. Campbell's operative orthopaedics. 8th ed. St Louis: Mosby-Year Book; 1992. p. 2749.
24. Clohisy JC, Akbarnia BA, Bucholz RD, Burkus JK, Backer RJ. Neurologic recovery associated with anterior decompression of spine fractures at the thoracolumbar junction (T12-L1). Spine (Phila Pa 1976) 1992;17(8 Suppl):S325-30.
25. Duh MS, Shepard MJ, Wilberger JE, Bracken MB. The effectiveness of surgery on the treatment of acute spinal cord injury and its relation to pharmacological treatment. Neurosurgery 1994;35:240-8.
26. Tator CH, Fehlings MG, Thorpe K, Taylor W. Current use and timing of spinal surgery for management of acute spinal surgery for management of acute spinal cord injury in North America: results of a retrospective multicenter study. J Neurosurg 1999;91(1 Suppl):12-8.
27. Tarlov IM. Spinal cord compression studies. III. Time limits for recovery after gradual compression in dogs. AMA Arch Neurol Psychiatry 1954;71:588-97.
28. Black P, Markowitz RS, Cooper V, Mechanic A, Kushner H, Damjanov I, et al. Models of spinal cord injury: Part 1. Static load technique. Neurosurgery 1986;19:752-62.
29. Mure PY, Galdo M, Compagnone N. Bladder function after incomplete spinal cord injury in mice: quantifiable outcomes associated with bladder function and efficiency of dehydroepiandrosterone as a therapeutic adjunct. J Neurosurg 2004;100(1 Suppl Spine):56-61.
30. Beric A, Light JK. Function of the conus medullaris and cauda equina in the early period following spinal cord injury and the relationship to recovery of detrusor function. J Urol 1992;148:1845-8.