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 1Orthopedics and Traumatology, 2Pharmacology and 3Pathology, 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 tavan mesanesi kullanarak akut spinal kord yaralanmasında erken cerrahi dekompresyonun yarımları değerlendirildi.

Gereç ve yöntemler: Bu çalışma, 9-12 aylık ve ortalamama ağırlıkları 2438 gr (dağılm; 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. Bütün tavanlar cerrahi işlemde sonraki yedinci günde sakrifiye edildi. Mesane dokusu izole edildi ve gevşeme-kasılma yanıtları alınmak üzere organ banyosuna asıldı.

Bulgular: Tüm deneylerden önce izole organ banyosuna 80 mM KCl eklenirken ve ortalamama ağırlıkları 2438 gr (dağılm; 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 gramlik kompresyon gücü uygulanıdı. Bütün tavşanlar cerrahi işlemde sonraki yedinci günde sakrifiye edildi. Mesane dokusu izole edildi ve gevşeme-kasılma yanıtları alınmak üzere organ banyosuna izole edildi.

Sonuç: Bu çalışmada akut spinal kord yaralanmasında erken cerrahi dekompresyonun yarımları değerlendirildi. Ancak erken ve gecikmiş dekompresyon grupları arasındaki farkın istatistiksel olarak anlamlı olmadığı bulundu.

Anahtar sözcükler: Erken dekompresyon; spinal cord yaralanması; omurilik; cerrahi; mesane.
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. Vertebreal fracture or dislocation is a frequent cause of spinal cord bruising or contusion.

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. 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.

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.

Most studies examining lower urinary tract function after SCI have used completely transected animals. Initially after thoracic spinal cord transection, the bladder is areflexic, causing urinary retention and bladder enlargement. Hyperreflexic bladder contractions re-appear after 7-10 days; voiding is possible but inefficient. A few studies have used partial compression or acute compression models such as the weight drop model. A few studies have used acute compression models such as the weight drop model. We performed SCI with the clip compression model. 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 °C. 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 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. 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 CaCl2, 22 mM NaHCO3, 1.2 mM MgCl2, 1.2 mM NaH2PO4, and 11.5 mM glucose. The solution was gassed with 95% O2 and 5% CO2 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.

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-
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.

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\cite{27} a weight drop technique\cite{28} a technique in which a weight compresses the cord by a device, similar to a technique by Black et al.\cite{28} constriciting the cord with a band\cite{29} and extradural clip compression injury by Rivlin and Tator\cite{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.\cite{27} Mure et al.\cite{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,\cite{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