Evaluation of Purinergic Mechanism for the Treatment of Voiding Dysfunction: A Study in Conscious Spinal Cord-injured Rats

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Background: To investigate the effect of a selective P2X3–P2X2/3 purinergic receptor antagonist (A-317491) on detrusor hyperreflexia in conscious chronic spinal cord-injured female rats.

Methods: Six chronic spinal cord-transected female Sprague-Dawley rats (290–336 g) were used in this study. Spinal transection at the T8–T9 segmental level was performed using aseptic techniques under halothane anesthesia. Fourteen to 16 weeks after spinal transection, A-317491, a selective P2X3 purinergic receptor antagonist, was administered intravenously in cystometry studies at increasing doses of 0.03, 0.1, 0.3, 1, 3, 10 and 30 µmol/kg at 40–50 minute intervals. Cystometrograms (CMGs) were performed before and after the administration of each dose of the drug.

Results: The continuous filling of CMGs revealed a large number of small-amplitude (>8 cmH2O), non-voiding contractions (NVCs) (average, 9.7 per voiding cycle) preceding voiding contractions (mean amplitude, 31 cmH2O; duration, 2.5 minutes), which occurred at an interval of 539 seconds and at a pressure threshold of 5.7 cmH2O. When tested in a range of doses (0.03–30 µmol/kg, intravenous), A-317491 in doses between 1 and 30 µmol/kg significantly (p < 0.05) increased the interval between voids by 25%, reduced the number of NVCs by 42–62%, and increased the pressure threshold for voiding by 53–73%, but did not change the amplitude of the duration of the voiding contractions. The effects of the drug were apparent within 10 minutes following administration.

Conclusion: These results indicate that purinergic mechanisms, presumably involving P2X3 or P2X2/3 receptors on bladder C-fiber afferent nerves, play an important role in the detrusor hyperreflexia that occurs after spinal cord injury in rats. [J Chin Med Assoc 2007;70(10):439–444]

Key Words: afferent nerve, detrusor overactivity, spinal cord injury, urinary bladder

There is no conflict of interest between the authors and the pharmaceutical company that provided the A-317491.

Introduction

Spinal cord injury (SCI) rostral to the lumbosacral level causes lower urinary tract dysfunctions, including detrusor hyperreflexia, incontinence, autonomic dysreflexia and detrusor-sphincter dyssynergia.1–6 Intravesical or systemic administration of capsaicin or resiniferatoxin in SCI cats and rats reduced detrusor hyperreflexia, uninhibited bladder contractions and vesicovascular reflexes, indicating that these disorders are triggered, at least in part, by capsaicin-sensitive C-fiber bladder afferents. These afferents also appear to contribute to neurogenic bladder dysfunction in humans, since the intravesical administration of capsaicin or resiniferatoxin in patients with SCI or multiple sclerosis decreases detrusor hyperactivity and the number of incontinence episodes.

In normal animals, a considerable proportion of bladder C-fiber afferents are chemosensitive but insensitive to mechanical stimuli (i.e. silent C-fiber afferents) and are, therefore, unresponsive to bladder distension.
Spinal cord transection and care of SCI rats
Six chronic spinal cord transected female Sprague-Dawley rats (290–336 g) were used in this study. Spinal transections at the T8–T9 segmental level were performed using aseptic techniques under halothane anesthesia. After a T9–T10 laminectomy, the dura matter was cut and the spinal cord transected with scissors, after which the point of a 16-gauge needle was moved 6 times around the inner surface of the exposed vertebra to ensure complete transection. Then, a piece of sterile sponge (Gelfoam; The Upjohn Company, Kalamazoo, MI, USA) was placed between the 2 cut ends of the spinal cord and the overlying muscle and skin were sutured. The rats were kept in a room in which the temperature was maintained at 23–23.5°C, and they were treated with antibiotics (ampicillin 150 mg/kg, intramuscular) every 2 days for 10 days.

The bladders were manually compressed 2 or 3 times daily after spinal transection to prevent over-distension of the bladder and infection. Perigenital stimulation with a cotton swab was used to activate a somatovesical reflex pathway and promote bladder emptying.13

Cystometric studies
Fourteen to 16 weeks after spinal transaction, cystometry was performed on the same animals. The rats were anesthetized with 2% halothane, and then the jugular vein was cannulated for drug and fluid administration. The bladder was exposed via a midline abdominal incision. The bladder end of a polyethylene catheter (inner diameter 0.76 mm, outer diameter 1.22 mm; Clay-Adams, Parsippany, NJ, USA) was heated to create a collar and passed through a small incision at the bladder dome. A 3-0 surgical silk suture (Ethicon, Somerville, NJ, USA) was tightened around the collar of the catheter. The cystostomy catheter was placed inside a larger polyethylene tube that acted as a tunnel to prevent the bending of the cystostomy catheter during the experiment. After the closure of the abdominal wound, the rats were placed in a restraining cage (Ballman Cage) in a normal crouched posture and allowed to recover from the halothane anesthesia for 1–2 hours before the experiment. The cystostomy catheter was connected to an infusion pump (model 55-4150; Harvard Apparatus, Holliston, MA, USA) to allow the continuous infusion of physiologic saline at room temperature into the bladder. Bladder pressure was monitored during cystometry by connecting the bladder catheter to a Statham pressure transducer. Continuous cystometrograms (CMGs) were performed using a constant infusion (0.2 mL/min) of saline into the bladder to induce repetitive voiding.14 The infusion of saline continued for at least 2 hours before the control voiding parameters were measured. Several control CMGs were performed on the same animal. Body temperature was monitored and maintained within the range of 36–38°C through an external heating device.

Drug administration
A-317491, a selective P2X3 purinergic receptor antagonist, was administered intravenously in the cystometry studies, at increasing doses of 0.03, 0.1, 0.3, 1, 3, 10 and 30 µmol/kg at 40–50 minute intervals. CMGs were measured before and after the administration of each dose of the drug.

Statistical analysis
The quantitative data are presented as mean ± standard error. Wilcoxon signed rank test was used to compare each parameter before and after drug administration.
A value of $p<0.05$ was taken to indicate a significant difference.

**Results**

**Control urodynamic measurements**

During the surgical procedure to insert the cystostomy catheter to conduct the CMGs, it was clear that all of the SCI rats exhibited severely distended and hypertrophied bladders. CMGs revealed a large number of small-amplitude (>8 cmH2O) non-voiding contractions (NVCs) (average, 9.7 per voiding cycle) during the saline infusion. Voiding contractions (mean amplitude, 31 cmH2O; duration, 2.5 minutes) occurred at an average interval of 539 seconds, equivalent to approximately 1.8 mL of infused saline between voids. The average pressure threshold for inducing voiding contractions was 5.7 cmH2O. The mean bladder capacity was calculated from the sum of the residual volume and infusion volume, which was 2.5 mL during CMGs. Repeated CMGs performed over 1–2 hour intervals in the same animal yielded reproducible filling and voiding parameters.

**Effect of A-317491 on voiding and bladder activity**

After obtaining control CMGs, A-317491 was administered intravenously in increasing doses, from 0.03 to 30 µmol/kg. The interval between voiding contractions (intercontractile interval) did not significantly change after smaller doses of the drug (0.03–0.3 µmol/kg), but increased significantly ($p<0.05$) after 1–30 µmol/kg of A-317491 (from 539 to 655–676 seconds) (Figures 1 and 2). The mean number of small-amplitude ($\geq$ 8 cmH2O) NVCs which occurred between the voiding contractions significantly decreased after 1–30 µmol/kg of the drug had been administered (from 9.72 to 5.6, 4.6, 4.75 and 3.63, respectively, $p<0.05$), but did not change significantly following smaller doses (0.03–0.3 µmol/kg) (Figures 1 and 3). The pressure threshold for inducing voiding increased after 1–30 µmol/kg of A-317491 (from 5.73 to 8.76, 9.91, 9.74 and 9.72, respectively, $p<0.05$), but was not significantly changed following smaller doses (Figures 1 and 4). The amplitude, duration of voiding contractions and baseline bladder pressure were not affected by any doses of the drug (Figures 5 and 6). The effect of A-317491 was apparent within 10–15 minutes after administration, and, after the largest dose of the drug, persisted for the duration of the experiment (2–3 hours).

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**Figure 1.** Cystometrograms showing the effects of A-317491 on voiding and bladder activity.

**Figure 2.** Intervals of effective contraction (intercontractile interval, ICI) before and after intravenous P2X3 receptor antagonist administration in cystometry study ($n=6$). *$p<0.05$.

**Figure 3.** Number of non-voiding contractions (NVCs) before and after intravenous P2X3 receptor antagonist administration in cystometry study ($n=6$). *$p<0.05$.
The present experiments revealed that the systemic administration of A-317491, a P2X3–P2X2/3 receptor antagonist, during continuous fast-infusion cystometry in conscious, chronic SCI rats reduced the number of non-voiding bladder contractions, increased the intra-vesical pressure threshold for inducing voiding and also increased the interval between voids without altering the amplitude or duration of the voiding contractions. The drug also decreased the frequency of voiding and increased the volume of urine per void without affecting total urine excretion under conditions of natural bladder filling in SCI animals held in a metabolism cage. These results indicate that purinergic mechanisms or P2X3 or P2X2/3 receptors on bladder afferent nerves play an important role in detrusor hyperreflexia due to SCI.

A-317491 is a non-nucleotide purinergic receptor antagonist that competitively blocks recombinant human and rat P2X3 and P2X2/3 receptors with a 100-fold selectivity over other types of P2X receptors. The drug exhibits very weak or no affinity for a large selection of other cell surface receptors and ion channels. Recent studies reveal that the drug also blocked native P2X3 and P2X2/3 receptors in rat dorsal root ganglion cells and dose-dependently reduced complete Freund’s adjuvant-induced thermal hyperalgesia (ED50 = 30 µmol/kg, subcutaneous), attenuating both the thermal hyperalgesia and mechanical allodynia (ED50 = 10–15 µmol/kg, subcutaneous) following chronic nerve constriction injury. These data, coupled with other reports indicating the presence of P2X2/3 receptors in IB4-positive C-fiber afferent neurons, indicate that purinergic mechanisms are involved in the peripheral and central sensitization of C-fiber nociceptors in chronic inflammatory and neuropathic pain. In the present experiments, similar doses of A-317491 reduced the frequency of voiding as well as the pressure threshold for inducing voiding without affecting maximal micturition pressure. This suggests that the drug suppressed the afferent limb of the spinal micturition reflex without altering the efferent limb of the reflex or the properties of the bladder detrusor muscle.

The CMG measurements in SCI female rats in the present study were similar to those obtained previously in awake SCI female rats. The most prominent changes following SCI were an increase in bladder capacity.
(2.5 mL vs. 1 mL in awake spinal cord-intact rats) and the presence of large numbers of NVCs prior to micturition. Although these changes were probably due to the spinal injury, it is also possible that some changes were related to the abdominal surgery and insertion of the bladder catheter on the day of the experiment or the fast infusion rate in cystometry.

The chronic transection of the thoracic spinal cord induces significant changes in the cystometric parameters of conscious female rats, including the appearance of uninhibited bladder contractions before the onset of micturition and obstructed voiding and decreased voiding efficiency. The present study reveals that A-317491 was effective in increasing the intercontractile interval and volume threshold, and in decreasing the number of NVCs in long-term SCI rats. However, the drug did not alter the amplitude and duration of voiding bladder contractions. This indicates that the drug did not alter the contractility of the detrusor muscle or the efferent pathways to the bladder.

Cockayne et al.12 reported that conscious or anesthetized P2X3-null mice also had significantly decreased micturition frequencies and increased bladder capacities, although their voiding bladder pressures were not reduced. These data suggest that the P2X3-null mice had a sensory rather than a motor defect in the bladder.

The rat bladders in this study were hypertrophied and enlarged following long-term SCI. It has been proposed that animals with a larger volume threshold tend to have a higher pressure threshold, indicating that the more distended bladders require a higher intramural tension to stimulate the afferent fibers, or that higher levels of afferent firing are needed to trigger a micturition reflex. However, the pressure threshold before treatment in this study was lower than that of the normal rats (5.73 vs. 13 cmH2O).16 This indicates that the micturition reflex is triggered more easily due to the increased excitability of the afferent nerves, despite the hypertrophied and enlarged bladders following SCI.

In the present study, CMGs were performed in conscious rats. It was reported that urethane affected the central transmitter mechanisms involved in the micturition reflex and decreased the voiding efficiency and volume threshold for inducing micturition in normal rats.17,18 Urethane was also reported to completely depress reflex micturition in chronic SCI rats.19 Thus, SCI rats appear to increase the sensitivity of their micturition reflex to urethane.20 Therefore, it would be better to test the CMGs in conscious awake animals in this study rather than urethane-anesthetized ones.

The bladder catheter was inserted through a dome in this study, which may have eliminated the impact of bladder outlet obstruction produced by a transurethral catheter.21 Nevertheless, the disadvantages of this transvesical catheterization method may be the possible irritation of the bladder, and the limitation of bladder movement during filling and voiding. However, this irritation change was not evident during the first day following transvesical catheterization. Therefore, this procedure is best for acute experiments immediately after catheter implantation22 or experiments pursued at least 6–7 days after implantation.23 Thus, the results of the CMGs that we performed on the same day as the transvesical catheterization in this study should be reliable.

Some previous studies measured the voided volume and residual volume, and subsequently calculated the bladder capacity and voiding efficiency24,25 of SCI rats. However, the measurements of voided volume and residual volume are relatively subjective, and it is difficult to measure these parameters accurately for conscious animals in the Ballman cage. Actually, Yoshiyama et al. also found that bladder capacity varied considerably from 0.33 to 3.3 mL within the SCI group.24 Nevertheless, in the present study, the rat bladders appeared consistently hypertrophied and enlarged in all animals. Thus, we did not measure the voided volume or the residual volume in this experiment.

Previous clinical studies reported that the intravesical administration of capsicain or resiniferatoxin produced clinical improvements in neurogenic bladder conditions, such as incontinence and detrusor hyperreflexia, in SCI and multiple sclerosis patients26–28 as well as hypersensitive bladder dysfunction in interstitial cystitis.30 This study revealed that A-317491 inhibited reflex bladder hyperactivity including non-voiding detrusor contractions without affecting voiding contractility, which is similar to the delayed suppressed effects elicited by intravesical capsaicin or resiniferatoxin administration. Thus, A-317491 may provide a new alternative treatment for the above pathologic conditions. The possibility of oral administration is the obvious advantage of A-317491 over intravesical instillation of capsicain or resiniferatoxin. In clinical terms, the intravesical administration of capsaicin can produce suprapubic pain, burning, macroscopic hematuria, or increased incontinence during the first week after treatment.30 Therefore, after conducting further clinical trials to validate the efficacy and side effects, A-317491 may be a better treatment of choice for neurogenic bladder disorders related to bladder afferent excitability, such as SCI, multiple sclerosis and interstitial cystitis.
References


