Original article

The ameliorating effect of dantrolene on the morphology of urinary bladder in spinal cord injured rats

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In animal models of spinal cord injury (SCI), the urinary bladder can undergo significant structural and physiological alterations. Dantrolene has been shown to be neuroprotective by reducing neuronal apoptosis after SCI. Furthermore, in addition to its anti-inflammatory and antioxidant properties, it appears to have a beneficial action on voiding, once this drug acts on the external urethral sphincter relaxation. In the present study, we investigated the effects of dantrolene on urinary bladder injury that follows experimental SCI. Forty-six male Wistar rats were laminectomy at T13, and a compressive trauma was performed to induce SCI. After euthanasia, the urinary bladder was removed for gross and histological evaluation. Traumatized animals showed urinary retention with severe hemorrhagic cystitis. Injured animals treated with dantrolene had less bladder hemorrhage and inflammatory infiltrate than those treated with placebo (p < 0.05). Our results demonstrate that dantrolene may protect against bladder lesions that follow SCI. Treating spinal cord-injured patients with this agent may be a promising additional therapeutic strategy to alleviate the accompanying inflammatory process. The results of the current study show that dantrolene has protective effects on spinal cord contusion-induced urinary bladder injury. The impaired integrity of bladder morphology was ameliorated by dantrolene treatment.

Introduction

Spinal cord injury (SCI) produces primary damage at the injured site that is followed by a delayed secondary lesion extending rostrocaudally, leading to progressive tissue destruction. The neurodegeneration induced by trauma is characterized by interruption of ascending and descending axons, loss of neurons and glia and demyelination, resulting in motor, sensory and autonomic functional deficits [7,13,27,28]. The SCI alters the complex neural circuits that contribute to the coordinate activity of the bladder and external urethral sphincter (EUS) and causes significant alterations in lower urinary tract function. Spinal lesions above the lumbar sacral level lead to inefficient voiding because the EUS contracts, while the bladder is contracting (detrusor-sphincter dyssynergia), which impedes voiding, leads to large residual urine volume and bladder over distention, predisposing to inflammation and hemorrhagic interstitial cystitis [2,18,25,28].

In recent years, much attention has been focused on secondary injury of SCI, which is an important potential target for therapeutic intervention. The development of any form of pharmacological therapy that can reduce or alleviate even some of the adverse outcomes associated with SCI has proven difficult due to the complexity of the injury [4,8,14,39]. Functional recovery of the lower urinary tract is important in patients with SCI to eliminate devastating urinary problems and improve quality of life [23,25,28,29].

Dantrolene is a drug that inhibits the ryanodine receptor Ca2+ channels (RyR) located on the sarco-endoplasmatic reticulum in skeletal muscle (RyR1) and neuronal cells (RyR3) [21,46]. It blocks calcium-induced calcium release from intracellular Ca2+ stores, preventing cytosolic Ca2+ overload [12,21,42]. Clinically, dantrolene is used as muscle relaxant and in the treatment of malignant hyperthermia [24,31]. It has been shown to possess antioxidant [9,41] and anti-inflammatory [11,19] properties.

Previous investigations have assessed its neuroprotective effects in several models of ischemic and traumatic brain injury [16,26,34,35,43], and traumatic [38], ischemia/reperfusion [22] and compressive model of SCI [40]. Moreover, there are evidences that this drug acts on the relaxation of the skeletal muscle of EUS, thereby reducing the resistance to bladder voiding and helping to control micturition [17,20,37]. However, to the best of our knowledge, there are no reports of the effect of dantrolene on the morphology of the urinary bladder after SCI. Therefore, the aim of the current study was to investigate the potential effects of...
dantrolene on urinary bladder injury that follows SCI in traumatized rats.

Materials and methods

This study was approved and performed in agreement with the Ethical Principles in Animal Experimentation, adopted by the Ethics Committee in Animal Experimentation from Federal University of Minas Gerais (CETEA/UFMG, protocol no. 059/03).

Animals and surgical procedure

Forty-six male Wistar rats aged 12 weeks and weighing 320–350 g were used in this study. Rats were kept under a 12/12 h light-dark cycle for 14 days of acclimation with commercial rodent food and water ad libitum. Pre-anesthetic medication was performed with tramadol (2 mg/kg, orally) and induction and maintenance was carried out with isoflurane administered by mask in a semi-opened system. The animals were positioned in prone position, prepared for aseptic surgery and received prophylactic antibiotic therapy with cephalothin (30 mg/kg, intravenous). Skin and subcutaneous tissue were incised in the dorsal midline extending from T6 to L1, the paravertebral muscles dissected and laminectomy of T13 was performed with the employment of a pneumatic drill. After visualization of the spinal cord covered by the intact dura, a compressive model of SCI was performed, as previously described [1,5,36], using a weight of 70 g/cm loading to the dorsal surface of the spinal cord. Afterwards, the site was irrigated with saline, the muscles approximated, and the reduced dead space and skin sutured using an unabsorbed suture. During anesthetic recovery, the animals were kept warm in a box heated approximately to 37 °C. They received tramadol (2 mg/kg, orally), every 8 h for three days. Abdominal massage was performed three times a day in all animals to assist with urination and defecation.

Treatment

The therapeutic protocol consisted of 10 mg/kg of dantrolene (Cristália Lab. Itapira, SP, Brazil) diluted in 15 ml of water for injection given in single dose, intraperitoneally 1 h after laminectomy. The control groups received only water for injection as placebo given in single dose, intraperitoneally 1 h after laminectomy.

Experimental groups

The animals were randomly divided into six groups according to the protocol of treatment and the time of euthanasia. GI (n = 7) underwent laminectomy followed by SCI, treated with placebo and euthanized after 32 h; GII (n = 7) underwent laminectomy alone, treated with placebo and euthanized after 32 h; GIII (n = 8) underwent laminectomy followed by SCI, treated with dantrolene and euthanized after 32 h; GIV (n = 8) underwent laminectomy followed by SCI, treated with placebo and euthanized after eight days; GV (n = 8) underwent laminectomy alone, treated with placebo and euthanized after eight days; and GVI (n = 8) underwent laminectomy followed by SCI, treated with dantrolene and euthanized after eight days. The study and its results were carried out by investigators who were blind to the experimental conditions.

Gross and light microscopy findings

The animals that underwent laminectomy alone (GII and GV) showed neither gross nor histological changes in their urinary bladders, while those subjected to SCI had bladder distension and hemorrhagic cystitis of varying intensity among the different groups. Histologically, at 32 h after SCI in GI and GIII, there were multifocal areas of hemorrhage in the muscle layers and lamina propria of the urinary bladder, and mixed inflammatory infiltrate with macrophages, lymphocytes and neutrophils was also seen. At eight days post-SCI, the bladders from GIV showed higher lesion intensity with inflammatory infiltrate and hemorrhage in all layers when compared with rats that received dantrolene which had inflammatory infiltrate predominantly consisting of macrophages in the lamina propria. Their morphological bladder features were similar to those of non-traumatized animals (Fig. 1).

The quantification of these morphological findings showed the amount of hemorrhage and inflammatory infiltrate in the urinary bladder. At 32 h after SCI, the animals that received placebo (GI) had significantly more hemorrhage (GI = 19%) than those that received dantrolene (GII = 8.5%) (p < 0.01). At 32 h, the non-injured animals showed no hemorrhage (GIII = 0%). At eight days, the dantrolene-treated animals showed recovery from the hemorrhagic process (GI = 0.6%) compared to the placebo-treated group (GIV = 15%) (p < 0.001) and appeared not different when compared to the non-injured animals (GV = 0%) (p = 0.05) (Fig. 2). However, although there was no significant difference in the inflammatory infiltrate between the traumatized groups at 32 h (GI = 17.7%; GIII = 12.4%), at eight days the inflammatory infiltrate was smaller in animals who received dantrolene (GVI = 6%) than in those who received placebo (GIV = 16%) (p < 0.05) (Fig. 3).

Discussion

The results of the current study show that dantrolene has protective effects on spinal cord contusion-induced urinary bladder injury. The impaired integrity of bladder morphology was ameliorated by dantrolene treatment.

Spinal cord injury produces severe deficits within the urogenital system. The majority of these deficits are the result of disruption of supraspinal input to the spinal cord and reorganization of intraspinal circuitry in response to injury [18]. Micturition is mediated by neural circuits that are located in the lumbosacral cord [33]. Spinal injury above the lumbosacral level damages descending
pathways that normally coordinate somatic motor (via pudendal nerve) and parasympathetic control (via pelvic nerve) of the lower urinary tract, altering primary afferent pathways to the lumbosacral cord and, thus, impairing lower urinary tract function [6,25].

In animal models of SCI, the urinary bladder can undergo significant structural, physiological and molecular alterations. In those animals, hematuria associated with a cellular inflammatory response and a breakdown of the uroepithelium lining the lumen of the bladder often occur as a result of bladder over-distention. This breakdown is initiated early after injury and is characterized by a loss of transepithelial resistance and enhanced permeability to both water and urea. These alterations result from inefficient voiding because the EUS contracts, while the bladder is contracting (detrusor-sphincter dyssynergia), which impedes voiding, leads to large residual urine volume and bladder over-filling, predisposing to inflammation and hemorrhagic interstitial cystitis [2,18,25,28,45]. For all these reasons, patients with SCI are at higher risk for bacterial cystitis, chronic bacterial infections within and under the uroepithelial layer, and bladder cancer [2,18,25].

While there are some treatments for controlling detrusor hyperreflexia, for example, antimuscarinic agents such as bethanechol and neurotoxins such as capsaicin and resiniferatoxin, detrusor-sphincter dyssynergia remains difficult to manage without catheterization or surgical interventions [29].

To achieve therapeutic benefits on spinal cord-injured patients, drugs that affect calcium homeostasis have been employed.
experimentally. Dantrolene, a ryanodine receptor antagonist, inhibits Ca2+ efflux from the endoplasmic reticulum to the cytosol, resulting in a documented neuroprotective effect [3,21,30,46].

Evidence for the neuroprotective effects of dantrolene via an antiapoptotic mechanism has been reported after experimental induction of neuronal death [26,31,34,43,44]. On the other hand, few studies have been performed to investigate the effects of dantrolene on SCI. Thorell et al. [38] examined the role of intracellular calcium in mediating posttraumatic abnormalities in axonal conduction and demonstrated that dantrolene improved electrophysiological recovery in an in vitro model of compressive injury to an isolated spinal cord dorsal column segment. Most recently, dantrolene afforded neuroprotection in a model of spinal cord ischemia/reperfusion injury induced by abdominal aortic occlusion in rabbits [22]. Torres et al. [40] showed that dantrolene decreased apoptosis and protected neurons in an in vivo model of compressive SCI in rats.

Thus, dantrolene was a promising option to be tested on the consequences of an in vivo traumatic SCI model, as it had not been evaluated in such a situation. It was expected that this drug would have a protective effect on the impaired urinary bladder following SCI. It was hypothesized to aid in micaturation control, ameliorating the dysuria between detrusor and EUS that develops following SCI [17], resulting in less morphological alterations.

The efficacy of dantrolene in treating inflammatory and anti-nociceptive disorders mediated by cytokines [19] and by arachidonic acid metabolites [11,15] has already been demonstrated. It was also proven that its antioxidant properties prevent lipid peroxidation and protect cells against the toxic effects of oxygen free radicals [9,10,41]. Furthermore, the action of this drug on the relaxation of the EUS has been previously reported and, considering that voiding is one of the natural protective mechanisms of the urinary system, it is possible that the action of dantrolene on the sphincter has major roles in this process [17,20,32].

Importantly, our findings showed significant improvement of the damaged urinary bladder tissue in rats with SCI that received dantrolene. It is worth noting that, to rule out the possibility that pressure from the manual expression of urine was the source of hematuria, in this study the sham animals experienced bladder expression as well, as performed by Herrera et al. [18].

Even though we have not performed urodynamic tests, we believe that our macroscopic and histological findings, suggestive of an amelioration of a possible dysynergia between bladder and EUS, may be due to the direct action of the drug against the inflammatory process in the bladder wall and/or the direct action of the drug on the EUS skeletal muscle relaxation allowing a facilitated voiding. Moreover, we can suggest the association of these possibilities.

In summary, we demonstrate here, for the first time, that systemically injected dantrolene ameliorates the urinary bladder damage that follows SCI. These findings suggest that dantrolene may provide a promising additional therapeutic strategy for the management of SCI and alleviate its consequences. Future investigation, such as concerning a long-term evaluation with urodynamic tests, must be done to elucidate the broad potential of this drug and the exact pathway by which dantrolene promotes those benefits.

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References


