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ORIGINAL ARTICLE

Renal function in a rat model of neurogenic bladder, effect of statins and phosphodiesterase-5 inhibitors

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Abstract
Purpose Neurogenic bladder is a common complication of several central nervous system injuries. Statins and phosphodiesterase-5 (PDE-5) inhibitors are reportedly beneficial in neural injuries and urinary system dysfunction. The effect of simvastatin, sildenafil and tadalafil on several renal function indices of an animal model of neurogenic bladder was investigated.
Methods Forty male rats were assessed in five equal groups. Dura mater and the cord were injured with an aneurysmal clamp at the level of T9–T10 in all rats except in sham group. The sham and control groups (treated by normal saline), simvastatin (4 mg/kg), sildenafil (5 mg/kg), and tadalafil (2 mg/kg) groups received treatment (i.p.) for seven consecutive days following injury. Renal system and motor functions were assessed at day 28 following injury. Data were analyzed by analysis of variance followed by the Student–Newman–Keuls post hoc test.
Results Simvastatin improved both the renal and the motor function compared with the control group. However, sildenafil and tadalafil could only improve the motor function but could not make any significant differences in renal indices in comparison with the control group.
Conclusion Statins can effectively improve the motor and renal functions in a condition of renal dysfunction in a rat model of neurogenic bladder. PDE-5 inhibitors could help to improve motor function, but are not helpful in renal function, at least in short time.

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Introduction

Several injuries to the central nervous system (CNS) may lead to neurogenic bladder and its complications [1]. Diseases of aging such as vascular events, CNS tumors and injury, and Parkinson disease are among the underlying causes in most countries [2]. Ignoring the complications of neurogenic bladder, particularly in early ages, results in urine stasis and injuries to the upper urinary system that lowers the quality of life [3]. Iranian population suffer from the high rate of CNS injuries due to history of the 8 year war, numerous car and job accidents, and natural disasters like prevalent earthquakes.

Currently, there is no cure for the deterioration of renal function following the occurrence of neurogenic bladder and supportive care is the main stream of health system attempts. Conventional methods like routine catheterization cause several complications including mechanical injury to the urethra and recurrent infections [4]. Surgical treatments like reconstruction surgeries have shown to be associated with stone formation, epithelial hyperplasia, and malignancies [5].

Statins are found to have anti-inflammatory [6] and neuroprotective [7] effects independent of their cholesterol lowering function. They also decrease renal inflammatory diseases through molecular pathways and declining oxidative stress [8] and the urinary stone formation [9]. They are introduced to improve the renal function particularly in spinal injuries to prevent urinary complication [10].

Phosphodiesterase-5 (PDE-5) inhibitors are broadly used in spinal injuries due to erectile dysfunction. Recently, they

are reported to be beneficial in patients with urine storage and voiding problems like benign prostate hyperplasia [11]. Mechanism of action of sildenafil on bladder muscles has been investigated [12]. Nitric oxide (NO), the pathway which PDE-5 inhibitors reinforce, regulates the urination reflex through modulation of afferent nerve routes [13].

Spinal injuries above the sacral region lead to neurogenic bladder and detrusor hyperreflexia [2]. There are several animal models mimicking such injuries in rats. Thoracic injuries have specifically been applicable [14] and show detrusor overactivity [15]. Here, we compare PDE-5 inhibitors and statins to evaluate their outcome on renal function after causing bladder dysfunction in a rat model of neurogenic bladder following spinal injury.

Materials and methods

Forty Sprague–Dawley rats weighing 200–250 g were investigated in this study. All animals were accommodated in standard cages with 12 h light providing environment in 22 °C and were nourished with standard rat pellets. They all entered the laboratory 1 week prior to beginning of the study to have environmental adaptation and were then investigated at the same time. The protocol was in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Ethics Review Committee for Animal Experimentation.

The animals were divided into five equal groups ($n = 8$). Sham group underwent surgery without any spinal injury and treatment. The control group had spinal injury and was treated by physiologic normal saline (i.p.) for seven consecutive days. The simvastatin (4 mg/kg), sildenafil (5 mg/kg), and tadalafil (2 mg/kg) groups received treatment i.p. for seven consecutive days following spinal injury.

To induce spinal injury, animals were anesthetized with ketamine (90 mg/kg) and xylazine (5 mg/kg), extra doses were administered if needed during the surgery. A midline incision was made in lower thoracic region of the dorsal vertebrae and skin, fascia, and muscles were excised to reach the vertebrae. Laminectomy procedure was carried out at the level of T9–T10 and spinal cord was exposed. Dura mater and the cord were injured with an aneurysmal clamp (Aesculap-Werke AG, Germany, Closing force: 107.00); fat tissue was replaced at the injury site. Muscle, fascia and skin were sutured. Animals were kept under warm environment till complete recovery. Prophylactic anti-biotic therapy was considered by cefazolin (150 mg/kg).

Animals were kept for 28 days. This time is sufficient for neurogenic detrusor overactivity to form and results in urine stasis. Bladders were emptied twice a day by gentle palpation till spontaneous voiding happened. At 28th day animals were kept in a metabolic cage for 24 h. Twenty-four hour urine volume and protein were measured. Serum creatinine and blood urea nitrogen (BUN) were also measured at the same day. The hind limb motor function was investigated at day 3, 7, 14, 21 and 28 using the Basso Beattie Bresnahan (BBB) protocol [16]. Thereafter animals were weighed and sacrificed with ether overdose. The wet bladder was extracted and weighed after emptying.

SPSS version 18 was utilized for statistical analysis. Analysis of variance (ANOVA) was used to find differences and post hoc test of Student–Newman–Keuls was conducted to find differences among groups. Data are presented as mean ± SEM. P value of <0.05 is considered significant.

Results

A one-way ANOVA indicated that the body weight of rats was significantly different between groups 28 days following the neurogenic bladder induction injury and renal dysfunction ($F_{(4,35)} = 112.6$, $P = 0.001$); (Table 1). Post-hoc analysis showed that the body weight was significantly higher in the sham group than in the other groups.

Bladder weight and urine volume of 24 h were also significantly different between groups 28 days following the neurogenic bladder induction injury ($F_{(4,35)} = 189.2$,

Table 1 Renal and urinary functions of rats at 28th days following the neurogenic bladder induction injury and renal dysfunction

	Control	Sham	Sildenafil	Tadalafil	Simvastatin
Volume 24 h (ml/day)	8.5 ± 0.3	9.9 ± 0.3*	9.1 ± 0.4	8.9 ± 0.4	9.8 ± 0.2*
Serum Cr (mg/dl)	0.64 ± 0.02	0.52 ± 0.02*	0.61 ± 0.02#	0.63 ± 0.02#	0.55 ± 0.02*
Serum BUN (mg/dl)	54.8 ± 3.3	21.1 ± 1.4*	49.7 ± 2.7#	52.8 ± 2.9#	45.9 ± 2.8*#
Urine protein (mg/24 h)	68.4 ± 3.1	23.2 ± 1.7*	65.8 ± 3.0#	63.9 ± 4.2#	52.2 ± 3.3*#
Bladder weight (mg)	985.7 ± 32.6	120.0 ± 5.0*	891.4 ± 32.4*#	921.7 ± 40.7#	535.0 ± 20.3*#
Body weight (g)	214.4 ± 2.4	264.3 ± 2.1*	214.9 ± 1.8#	214.7 ± 2.9#	216.9 ± 1.7#

Data are expressed as mean ± SEM

*, # Different from the control and sham groups respectively, $P < 0.05$

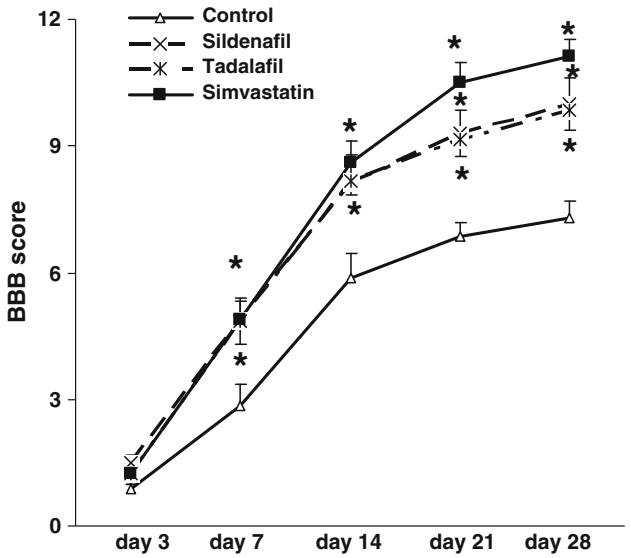

Fig. 1 Basso Beattie Bresnahan (*BBB*) score at days 3, 7, 14, 21 and 28 following the neurogenic bladder induction injury. Data are expressed as mean ± SEM. *Different from the control group, $P < 0.05$

$P = 0.001$; $F_{(4,35)} = 4.3$, $P = 0.007$, respectively); (Table 1). Bladder weight was low in the sham group than the other groups and also low in the sildenafil and simvastatin treated groups than control rats. Urine volume of 24 h was only higher in simvastatin treated and sham groups than in the controls.

Urine protein, serum BUN and serum creatinine significantly differed between groups 28 days following the neurogenic bladder induction injury ($F_{(4,35)} = 39.4$, $P = 0.001$; $F_{(4,35)} = 27.9$, $P = 0.001$; $F_{(4,35)} = 5.954$, $P = 0.001$, respectively); (Table 1). They were lower in the sham group than the other groups and also lower in the simvastatin treated group than the controls.

Motor function tests showed improvement in all groups as compared with control rats at the day 28 (Fig. 1). The most increasing in BBB score was found in the simvastatin treated group. The simvastatin, sildenafil and tadalafil treated groups showed significant improvement from the day 7. BBB score in sham group was 21 during 28 days following the neurogenic bladder induction injury.

Discussion

With regards to the results of this study, statins (such as simvastatin) improve the renal and urinary system function after injury to the CNS leading to neurogenic bladder in rats. PDE-5 inhibitors (such as sildenafil and tadalafil) along with simvastatin improve behavioral function of the hind limb after spinal injury but could not protect the animals from renal dysfunction.

The 24 h urine volume notably decreased in neurogenic bladder. Interestingly, simvastatin increased the volume at day 28 to levels very close to the sham group. The same result is reported by Shunmugavel et al. [10]. Serum creatinine also decreased very close to the sham group, however, serum BUN and 24 h urine protein level decreased but stayed in significant difference with the sham group. It seems that serum creatinine could be a better index of renal function in neurogenic bladder of rats, it reaches normal levels earlier. With regards to bladder weight increase up to eight times in the group with neurogenic bladder and no treatment, and significant body weight decline, it could be concluded that tissue injuries could be due to anatomic disruptions in the urinary system, urine retention and the following glomerular injury [17], leading to abnormal 24 h protein measurements and nitrogen loss at day 28. Bladder hyperplasia (eight times the normal bladder) may be due to neuronal signals causing muscular hypertrophy after spinal injury [18]. Those changes end in detrusor hyperreflexia and cause symptoms of neurogenic bladder such as urine stasis and disrupted renal function indices. Our results support employing the rat model of neurogenic bladder as it relevantly shows the changes expected in neurogenic bladder.

Tadalafil, as a long acting PDE-5 inhibitor, did not show any significant difference compared with the sildenafil treated group. Therefore, the dosage of sildenafil administration in this study with regards to similar studies [19] has the same results if given in seven consecutive days, even though it has shorter period of action. The dosage of simvastatin in this study was previously shown to have effects in animal models and higher doses had toxic effects [10].

There are studies showing the protective effects of simvastatin in proteinuria and renal system function in renal insufficiency [20]. Here, we found the same results in the rat model of neurogenic bladder and the following renal dysfunction. This could be due to direct effect of simvastatin in mesangial cells and glomerular regeneration [21]. Moreover, simvastatin's effect in decreasing the oxidative stress and its anti-inflammatory role should be considered [6]. Epithelium damage and urine exposure to bladder muscles as a foreign agent that causes inflammation plays an important role in stone formation and urinary dysfunction [22].

Although the hind limb motor function improved in all groups, none could reach the normal levels (sham group) and animals still suffered from disabling motor dysfunction. The simvastatin treated group showed functional recovery earlier than the other groups. However, all groups had significant improvement at the end of the 1st week. The same results are reported in previous studies for statins and PDE-5 inhibitors [10, 19]. However, there is no direct

relation defined between motor and renal function improvement.

PDE-5 inhibitors increase the intracellular concentration of cGMP, therefore augmenting the NO effects. Regarding the role of NO in regulation the neuronal pathways of voiding function [13, 14], PDE-5 inhibitors could improve the storage and urination function due to interfering action with non-functional contractions of a neurogenic bladder. Their beneficial outcomes in benign prostatic hyperplasia are previously observed [11, 12]. However, in our study they did not improve the renal function in a rat model of neurogenic bladder.

Statins can effectively improve the motor and renal functions in a rat model of neurogenic bladder. They can prevent serious injuries to the upper urinary tracts and therefore prevent deterioration of renal function indices. PDE-5 inhibitors could help to improve motor function, but are not helpful in renal function, at least in short time. Due to the limitation of this study which investigates several outcomes in an animal model but not in a human clinical state, there could be studies assessing for similar conditions in humans for the same conventional medications employed in this study.

According to our results, despite the significant motor function progression at day 28 in all groups, only the simvastatin treated group could manage to show better renal function indices. In general, with regards to aforementioned mechanisms suggested for statins' effects, it could be concluded that in short time following the appearance of urine stasis after neurogenic bladder condition, statins with possible anti-inflammatory and direct regenerative effects on renal tissues play a very important role in renal function improvement. However, regarding the simultaneous neural regeneration and motor function improvement in all groups, their additional beneficial effects on renal function recovery could not be ignored; but they are not solely beneficial and it is important to consider them in combination with other interventions. On the other hand, motor function improvement cannot independently provide positive effects on renal and urinary system function, at least in short time.

Conflict of interest None.

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