Naftopidil improves locomotor activity and urinary frequency in rats with pelvic venous congestion

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(Received 5 February 2016; and accepted 18 May 2016)

ABSTRACT

The $\alpha 1D/A$ receptor antagonist, naftopidil, inhibits micturition reflex by acting on various different sites. We examined the effects of naftopidil on bladder activity and changes in the induced urinary frequency using female rats with pelvic venous congestion (PC). Twenty-four female rats were divided into sham, PC, and PC/naftopidil groups. After anesthetizing rats in the PC and PC/naftopidil groups, the bilateral common iliac veins and uterine veins were ligated. Rats in the sham and PC groups were fed a standard diet, while rats in the PC/naftopidil group were fed diets containing 0.04% naftopidil. After 4 weeks of treatment, locomotor activity, urinary nitric oxide metabolites (NOx), continuous cystometry, and plasma monoamine measurements were performed. PC rats exhibited a decrease of locomotor activity, a shorter interval between bladder contractions on continuous cystometry, and decreased urinary NOx and plasma serotonin levels than the sham rats. The PC/naftopidil rats exhibited an increase of locomotor activity, a longer interval between bladder contractions, and increased urinary NOx and plasma serotonin levels. Therefore, naftopidil might improve bladder dysfunction induced by pelvic venous congestion due to several actions in the central nervous system and bladder tissue, as well as acting as an $\alpha 1$ blocker to cause pelvic venous dilation.

Unilateral or bilateral ovarian vein incompetence can cause pelvic congestion syndrome, which induces chronic pain, irritable bladder, dysfunction of the pelvic organs, or vulval varices and varicose veins in the lower limbs (1, 5, 7). Our previous study demonstrated that chronic prostatitis and stress urinary incontinence were related to the existence of vena cava reflux caused by tricuspid regurgitation (28). When we examined a female case with pelvic congestion syndrome using external iliac venography, we found that pelvic venous congestion occurred with tricuspid regurgitation and without pelvic

venous valves (25). Our previous studies have also demonstrated that ligation of the bilateral common iliac veins in male rats or ligation of the bilateral common iliac veins and uterine veins in female rats induced changes in the urinary frequency and low locomotor activity (30). As compared to the intact rats, these surgically altered rats exhibited a bladder blood flow decrease that was about 80% of the flow in the intact bladder. Therefore, pelvic venous congestion may be one of the causes of pelvic ischemia, and might be related to several lower urinary tract symptoms/diseases.

When examining hypertension, the relevance of lower urinary tract symptoms and their association with hypertension need to be taken into consideration. Spontaneously hypertensive rats have been shown to have a significantly lower bladder capacity and voiding volume (24), and these rats also exhibit

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a lower prostate blood flow and have an increased prostate size (23). In the atherosclerosis-induced chronic bladder ischemia rat model, the resulting detrusor overactivity leads to an increase in the voiding frequency (18). Since atherosclerosis is thought to be a risk factor for benign prostatic hyperplasia (BPH), it has been suggested that there is a relationship between high vascular resistance and the development of BPH (12, 23). Therefore, it is thought that the hypertension and/or arteriosclerosis that reduces arterial blood flow and subsequently causes ischemia will induce BPH or overactive bladder. *In vitro* studies using human prostate tissue have demonstrated that smooth muscle tone is mediated by alpha 1A-adrenoceptors, which are abundant in the prostate and the bladder neck. Consequently alpha 1-adrenoceptor antagonists have become the first-line treatment for the relief of BPH symptoms (11).

Naftopidil, the alpha 1A/D-adrenoceptor antagonist, has been widely used to BPH because it reduces resistance in the prostatic urethra (31). Multiple studies using rats have shown that naftopidil inhibits the micturition reflex after oral administration (9, 22), intravenous injection (2), injection into the medial frontal lobe (14), and after injection into the subarachnoid space at the lumbosacral cord level (26). As naftopidil can inhibit the release of adenosine triphosphate (ATP) from the bladder epithelium (8, 27), it may facilitate the afferent nerves and evoke the micturition reflex. Clinical studies that have examined the various agents that can improve urine storage symptoms have demonstrated that naftopidil exhibits a clear superiority over the other α 1A blockers (6). This action may be related to multiple effects on the cerebrum, spinal cord, and bladder epithelium. Therefore, our current study investigated the mechanism of naftopidil's efficacy on the bladder and locomotor activity in pelvic venous congestion rats with induced changes in urinary frequency and low locomotor activity.

MATERIALS AND METHODS

Animals. A total of 24 female Sprague-Dawley rats weighing 195–230 g were used in the study. To create the pelvic venous congestion (PC) animals, 16 rats were first anesthetized with 2% isoflurane. After an incision was made in the lower abdomen, the bilateral common iliac veins were ligated with metal clips, while the bilateral uterine veins with uterine arteries and uterine horn were ligated en bloc at a site near the ovaries. After ligating the veins, the distal common iliac veins became dilated. After closing

the abdomen, all animals were given a subcutaneous injection of an antibiotic (30 mg of ampicillin). The 16 rats in this group were then randomly divided into the PC and the PC/naftopidil group ($n = 8$ in each group). For the sham group, 8 additional rats were anesthetized with isoflurane, and the bilateral common iliac veins were dissected free of the common iliac arteries. Rats in the sham and PC groups were fed a standard diet, while rats in the PC/naftopidil group were fed powder diets that contained 0.04% naftopidil (Asahi Kasei Pharma Corporation, Tokyo, Japan). At 4 weeks after the operation and after all of the rats in the 3 groups underwent the different experimental protocols in turn (described in detail in the text that follows), the data were compared and analyzed among the 3 groups. This study protocol (No.5803) was approved by the President of the University of the Ryukyus based on the judgment of our institutional Animal Care and Use Committee.

Locomotor activity. We measured locomotor activity as a surrogate marker of pelvic pain. Rats were housed individually in plastic cages with woodchip bedding, food, and water. Locomotor activity was measured using a digital counter and an infrared sensor (NS-ASS01; Neuroscience, Inc., Tokyo, Japan). Room lights were on from 8 : 00 A.M. to 8 : 00 P.M. Therefore, locomotor activity during the dark period (active phase in rats) was calculated as the sum of all movements from 8 : 00 P.M. to 1 : 00 A.M., which is the period that is least affected by the switching of the light on and off.

Urinary NOx measurements. After locomotor activity assessment, spontaneous voided urine was collected for each rat for measurements of NOx (nitrite and nitrate) and creatinine. Urinary NOx is mainly secreted from bladder epithelial cells, and inhibits bladder afferent nerve activity or bladder contractions (20). NOx were measured by using the Griess method. Briefly, after a urine sample was deproteinized by the addition of an equal volume of methanol, the sample was analyzed using an automated NO detector high-performance liquid chromatography system (ENO-20; Eicom, Kyoto, Japan).

Cystometry. Rats were anesthetized with urethane (0.6 g/kg subcutaneously) and placed in a restraining cage (NAIGAI-CFK-1P; NMS, Tokyo, Japan), after which a polyethylene catheter (PE50; Clay Adams, NJ, USA) was inserted transurethrally into the bladder. This catheter was then connected to an infu-

sion pump and pressure transducer via polyethylene tubing and the bladder was filled with physiological saline at a rate of 0.05 mL/min. Continuous cystometry was performed for at least 90 min, during which time the bladder activity was recorded.

Plasma monoamines measurements. After cystometry, blood was collected from the inferior vena cava for evaluating the balance of the autonomic nervous system. Plasma was obtained by centrifugation and plasma monoamines levels were measured using a standard HPLC method in a contract laboratory (BML Inc, Tokyo, Japan).

Statistical analysis. Results are reported as the mean $\pm$ standard error of the mean (SEM). Student's *t*-test for unpaired data was used for statistical analysis where appropriate, with $P < 0.05$ considered to indicate statistical significance.

RESULTS

Locomotor activity

During the dark period, locomotor activity ($5,291 \pm 314$ movements) in the PC group was significantly lower than in the sham group ($6,361 \pm 464$ movements) ($P < 0.05$). Locomotor activity ($8,450 \pm 541$ movements) in the PC/naftopidil group was significantly higher than that observed in the sham and the PC groups ($P < 0.001$, respectively). Since visceral pain activates the mesocorticolimbic dopamine system which induces the suppression of locomotor activity, the increased locomotor activity suggests a reduction of pain by naftopidil.

Urinary NOx

The urinary level of NOx corrected for creatinine (cre) (urinary NOx/cre ratio) was significantly lower in the PC group (4.4 ± 1.3 $\mu\text{M}/\text{cre}$) versus the sham group (8.1 ± 0.8 $\mu\text{M}/\text{cre}$) ($P < 0.05$). The urinary NOx/cre ratio (15.8 ± 2.1 $\mu\text{M}/\text{cre}$) in the PC/naftopidil group was significantly higher than that observed in the sham and PC groups ($P < 0.001$, respectively) (Fig. 1). The decrease of urinary NOx/cre ratio in the PC group is thought to be the suppression of bladder epithelial activity by pelvic congestion, and that the increase of urinary NOx/cre ratio in the PC/naftopidil group suggest to promote the NOx secretion from bladder epithelium by naftopidil.

Continuous cystometry

The interval between bladder contractions in the PC group (8.2 ± 0.6 min) was significantly shorter than

Fig. 1 Comparison of NOx urinary levels corrected for creatinine (cre). The urinary NOx level was significantly lower in the PC group versus the sham. The urinary NOx level in the PC/naftopidil group was significantly higher than that observed in both the sham and PC groups. * $P < 0.05$, ** $P < 0.01$, $n = 8$.

that in the sham group (14.2 ± 1.0 min) ($P < 0.001$). In contrast, the interval between the bladder contractions in the PC/naftopidil group (14.0 ± 2.6 min) was significantly longer than that observed in the PC group, and almost the same as that for the sham group ($P < 0.05$). There were no significant differences for either the bladder baseline pressure or the maximum bladder contraction pressure among the 3 groups (Fig. 2). The shorted or prolonged intervals between bladder contractions without changes of bladder baseline and contraction pressure by pelvic venous congestion or naftopidil suggest that pelvic venous congestion and naftopidil mainly affect bladder afferent nerve activity and/or spinal afferent pathways but not bladder efferent nerve activity nor the bladder smooth muscles.

Plasma monoamines levels

Serotonin plasma levels were significantly lower in the PC group (455 ± 154 ng/mL) versus the sham group (1287 ± 273 ng/mL) ($P < 0.05$) (Fig. 3). Serotonin plasma levels ($1,165 \pm 214$ ng/mL) were significantly higher in the PC/naftopidil group versus the PC group (455 ± 154 ng/mL) ($P < 0.05$) (Fig. 3D). Plasma levels of adrenaline ($7,559 \pm 1,756$ ng/mL) and dopamine (71 ± 11 ng/mL) were significantly lower in the PC/naftopidil group as compared to the sham group ($16,773 \pm 3,319$ ng/mL and 124 ± 13 ng/mL; $P < 0.05$ and $P < 0.01$, respectively). Noradrenaline plasma levels were neither affected nor statistically significant for either the PC or naftopidil groups. The decreased serotonin levels in the PC group suggest the depressed condition maybe by pelvic pain. In our previous study using male patients

Fig. 2 Comparisons of the continuous cystometric parameters. The interval between the bladder contractions in the PC group was significantly shorter than that in the sham group (A). In contrast, the interval between the bladder contractions in the PC/naftopidil group was significantly longer than that in the PC group, and it was almost the same as that observed in the sham group (A). There were no significant differences among the 3 groups for the bladder baseline pressure (B) or for the maximum bladder contraction pressure (C). * $P < 0.05$, ** $P < 0.01$, $n = 8$.

Fig. 3 Changes in the plasma monoamines levels. Plasma adrenaline (A) and dopamine (C) levels were significantly decreased in the PC/naftopidil versus the sham group. The plasma serotonin level (D) was significantly lower in the PC group versus the sham group, while it was significantly higher in the PC/naftopidil group versus the PC group. There were no significant differences among the 3 groups for the plasma noradrenaline level (B). * $P < 0.05$, ** $P < 0.01$, $n = 8$.

with benign prostatic hyperplasia (10) or intact female rats (28), naftopidil decreased plasma catecholamine levels and increased serotonin levels. In the present study, naftopidil also showed same results even in rats with pelvic venous congestion.

DISCUSSION

Our previous studies demonstrated that pelvic venous congestion decreased the bladder blood flow and increased the bladder vascular permeability (15). When we administered carbazochrome sodium sul-

fonate hydrate (carbazochrome), which is a stable oxyepinephrine derivative that acts as a capillary stabilizer, in pelvic venous congestion rats, the continuous cystometry test indicated that there was an increased interval between the bladder contractions. Furthermore, when plasma was added dropwise to the bladder in a rat model, the maximum bladder contraction pressure increased, while the interval between the bladder contractions shortened (13). In a tranilast-induced interstitial cystitis rat model, carbazochrome increased both the locomotor activity and the interval between the bladder contractions (16),

similar to our current results that were found for naftopidil in the pelvic venous congestion rats. Therefore, vascular permeability increases due to pelvic venous congestion and subsequent extravascular leakage of a stimulatory substance might be one of the factors that contribute to the occurrence of lower urinary tract symptoms. Thus, the increase of the locomotor activity in the pelvic venous congestion rats that was observed in our current study suggests that naftopidil may have both a capillary stabilizing action, and inhibit the extravascular leakage of stimulatory substances that act on the bladder.

In the atherosclerosis-induced chronic bladder ischemia rat model, changes in the urinary frequency were shown to be associated with an increase of oxidative stress markers in the urine or bladder tissue (17, 19). Furthermore, atherosclerosis-induced chronic ischemic bladder also decreased constitutive nitric oxide synthase (NOS) (20). Since reports have shown that nitric oxide has both relaxatory and facilitatory effects, as well as having direct effects on the detrusor and modulatory effects via the afferent nerves, it has been suggested that the effect of the nitric oxide on the afferent nerve signaling occurs primarily in an inhibitory fashion (4). The decrease of urinary NOx in the pelvic venous congestion rats examined in the present study might indicate that bladder tissue hypoxia is one of causes of the alterations in the urinary frequency. Since administration of naftopidil reversed the level of the urinary NOx, this suggests that naftopidil acted as an α -1 blocker and partially improved the bladder tissue hypoxia by causing relaxation of the pelvic vessels. Saito *et al.* reported that bladder blood flow in the spontaneously hypertensive rat is significantly lower than that in the normotensive Wistar rat, and that vasodilators such as nicorandil (K_{ATP} channel opener and NO donor) increase the bladder blood flow in the spontaneously hypertensive rat (21). Furthermore, treatment with naftopidil increased the bladder blood flow in a dose-dependent manner in the spontaneously hypertensive rat group (22). These results may also support our data which relationships between bladder blood fluid and NOx.

Our previous study using rats, our clinical study using BPH patients, and our present study have all shown that the administration of naftopidil decreases the plasma adrenaline and/or dopamine levels and increases the plasma serotonin levels (10). Naftopidil inhibits bladder contractions via spinal glycinergic, GABAergic, and serotonergic inhibitory mechanisms, while noradrenaline activates bladder contractions via spinal excitatory glutamatergic mechanisms (29).

Moreover, injections of naftopidil into the medial frontal lobe inhibit bladder contractions (14). When rats were given an intravesical infusion of acetic acid in order to stimulate the bladder, we also found that naftopidil administration decreased the urinary ATP levels in these animals (27). ATP has been shown to be one of the stimulating substances that are secreted from the bladder epithelium (3). Therefore, these previous results suggest that naftopidil acts comprehensively on several sites such as the frontal lobe, spinal cord and bladder epithelium via monoaminergic and amino acids neuronal mechanisms as well as increasing NO synthase, decreasing ATP secretion and inhibiting the appearance of the micturition reflex. In addition to the above described actions for naftopidil, it also increases the level of urinary NOx. Thus, it is possible and perhaps more likely that naftopidil acts as an α -1 blocker, thereby inducing venous dilation and causing the development of venous collaterals. At the present time, however, the mechanisms by which naftopidil improves pelvic venous congestion has yet to be definitively elucidated.

In conclusion, pelvic venous congestion induced a decrease of locomotor activity, a shorter interval between bladder contractions as observed by continuous cystometry, and decreased both the urinary NOx and plasma serotonin levels. When naftopidil was administered in these rats, there was an increase of the locomotor activity, a longer interval between the bladder contractions during cystometry, and increases of both the urinary NOx and plasma serotonin levels. This is the first report that naftopidil increased the urinary NOx. Moreover, naftopidil also decreased both the plasma adrenaline and dopamine levels in comparison to the sham rats. Thus, naftopidil might improve bladder dysfunctions due to pelvic venous congestion not only via actions on the central nervous system and bladder tissue, but also by acting as an α -1 blocker and causing pelvic venous dilation.

CONFLICT OF INTEREST STATEMENT

This study was supported by Asahi Kasei Pharma Corporation.

ETHICAL STANDARDS

In this research, study protocol was approved by the President of the University of the Ryukyus based on the judgment of our institutional Animal Care and Use Committee.

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