Nicorandil, a Potassium Channel Opener and Nitric Oxide Donor, Improves the Frequent Urination without Changing the Blood Pressure in Rats with Partial Bladder Outlet Obstruction

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Objective: It was studied to determine if nicorandil can improve frequent urination in rats with partial bladder outlet obstruction (BOO) without changing the blood pressure. Materials and methods: Voiding behavior was observed 6 to 8 d after obstruction in female rats with BOO that loaded 30 ml/kg of water. A drug was administered orally. Changes in systemic blood pressure and heart rate were studied in conscious BOO rats using the tail cuff method. Results: The voiding frequency was increased and the average voided volume was decreased in BOO rats compared with normal rats. Nicorandil (1 mg/kg), cromakalim (0.1 mg/kg) and isosorbide dinitrate (ISDN; 1000 mg/kg) decreased voiding frequency significantly in BOO rats. Nicorandil also increased the average voided volume significantly. Although cromakalim and ISDN at doses effective at decreasing voiding frequency caused blood pressure to drop, nicorandil at an effective dose did not affect blood pressure and heart rate. Conclusion: Nicorandil improved frequent urination without changing the blood pressure. These results suggested that a hybrid of a K_{ATP} channel opener and nitric oxide donor, nicorandil was bladder-selective compared with vasculature in BOO rats.

Key words nicorandil; K_{ATP} channel; nitric oxide; overactive bladder; benign prostatic hyperplasia

With advance of an aged society, the populations with lower urinary tract symptoms (LUTS) are increasing. These symptoms can divide into problems with storage (urinary frequent urination, nocturia and urgency) and emptying (incomplete emptying and development of urinary retention). The frequent urination is one of severe urological symptoms caused by the lower urinary tract dysfunction such as overactive bladder (OAB) and benign prostatic hyperplasia (BPH) resulting in bladder outlet obstruction (BOO). Antimuscarinics are the first line treatment at present and show symptom improvement in many patients with frequent urination[1] but are limited by side effects such as dry mouth, blurred vision, constipation and urinary retention in some patients, resulting in a significant level of noncompliance. Therefore, there are substantial unmet medical needs for novel or more effective agents compared with antimuscarinics. Currently, a number of drugs with a variety of mechanisms are in development.

ATP-sensitive potassium channel openers (KCO) and nitric oxide (NO) donors have been possible candidates with novel mechanism for the treatment of frequent urination. Opening the potassium channel in bladder smooth muscles evokes membrane hyperpolarization which reduces calcium influx *via* a voltage-dependent calcium channel and relaxes the detrusor smooth muscle. Expression of ATP-sensitive potassium channel has been detected in bladder from several species including human and rat.[2] KCO such as pinacidil and cromakalim have been shown to inhibit isolated bladder contractions in several species, including humans.[3—5] Although cromakalim causes an increase in micturition interval in rats, blood pressure was reduced at the same dose.[6] On the other hand, the important action of NO on outflow regions appears to be widely accepted.[2] Masuda *et al.* reported that endogenous and exogenous NO depresses reflex bladder activity by suppressing the excitability and/or release of transmitters from bladder afferent nerves in tests using overactivity of the detrusor induced by capsaicin.[7] Thus, we postulated that a drug that possesses nitrate activity in addition to KCO activity, as well as the bladder selective characteristics, would be more useful in the treatment of frequent urination than a pure KCO.

Nicorandil (2-nicotinamidoethyl-nitrate ester), which has been used clinically as treatment for angina and acute heart failure, is a KCO that donates NO. Nicorandil is known to improve coronary circulation without changing the systemic blood pressure in humans.[8] The pharmacological effects of nicorandil on frequent urination in three different OAB models in rat were reported recently.[9] This study was conducted to confirm whether nicorandil could improve the frequent urination symptom without changing the blood pressure using the BOO rat model, compared with those of a pure KCO, cromakalim and a NO donor, isosorbide dinitrate (ISDN).

MATERIALS AND METHODS

Tests were conducted on female SD rats (140—200 g, Japan SLC, Inc., Shizuoka, Japan). All rats were fed an ordinary laboratory chow and allowed free access to water under a constant light and dark cycle of 12 h. All animal procedures were conducted in accordance with Chugai Pharmaceutical's ethical guidelines for animal care, and all experimental protocols were approved by the Animal Care Committee of the institution.

For the model of BOO our methods were adapted from those of Malmgren *et al.*[10] Briefly, the female SD rats were

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anaesthetized with isoflurane and the bladder and urethra were exposed through a midline abdominal incision. A 1.2 mm diameter stainless steel rod was placed parallel to the urethra, and a silk ligature was tied around the proximal urethra and the rod. The abdominal musculature and the skin were closed after the rod was removed. At the beginning of each experiment animals that showed signs of urinary overflow incontinence (urine wetness around the external genitalia) were excluded from the study.

Voiding behavior at 6 to 8 d after surgery was measured by the modified methods of Hashimoto *et al.*[11] Briefly, voiding behavior was measured in a metabolic cage and the volume of urine was measured as the change in weight of a collection tube on micro balance. The output of the balance was input to a urinary output measurement system (Ver. 1.02, Muromachi Kikai Co., Ltd., Tokyo, Japan). A 30 ml/kg volume of water was administrated orally following an acclimation period that lasted 30 min after the animals were placed in a cage. Vehicle or the test drug was administrated orally at a volume of 30 ml/kg following a voiding measurement period of 3 h after water loading (pre-drug session) and a rest period that lasted 2 h. Voided volume was then measured for another 3 h (drug treatment session). Immediately after the drug treatment session was completed, retained urine in the bladder (residual urine) was collected under deep pentobarbital-Na anesthesia (100 mg/kg), and then its weight was measured. The entire bladder was dissected out and weighed to confirm the severity of urethral obstruction.

Systolic blood pressure and heart rate were measured by a tail cuff and pneumatic pulse transducer (UR-5000, Muromachi Kikai Co., Ltd., Tokyo, Japan) at pre-administration and 30 min after vehicle or 1 test drug (30 ml/kg) were administered orally to conscious BOO rats.

Nicorandil and cromakalim were synthesized in our organic chemistry laboratory and ISDN was purchased from Sigma-Aldrich. Nicorandil was dissolved in distilled water. Cromakalim and ISDN were suspended in 3% gum arabic solution and given to animals orally at a volume of 30 ml/kg.

Results are expressed as mean±S.E.M. of 5—10 rats. Significance of differences was analyzed using Student's *t* test or Dunnett's test in voiding behavior and paired *t* test in the hemodynamics. Probability values less than 0.05 were considered significant. Statistical analysis was performed using SAS 8.02 (SAS Institute, Cary, NC, U.S.A.).

RESULTS

When measuring voiding frequency and voided volume before the each drug administration (pre-drug session), partial obstruction of urethra in rats caused an increase in voiding frequency (3.4±0.3 to 18.6±1.4 n/3 h) and decreased the voided volume (1.15±0.12 to 0.13±0.01 ml/void). No significant differences were detected in the voiding frequency, and voided volume between any BOO group. Although the bladder weight of BOO rats measured after experiment were less than 3 times heavier than that of non-BOO rats (83.2±6.0 *vs.* 234.4±18.2 mg), there were no significant differences among the BOO groups. The parameters for voiding in the BOO group treated with vehicle (control group) did not change noticeably between the pre-drug and drug treatment sessions.

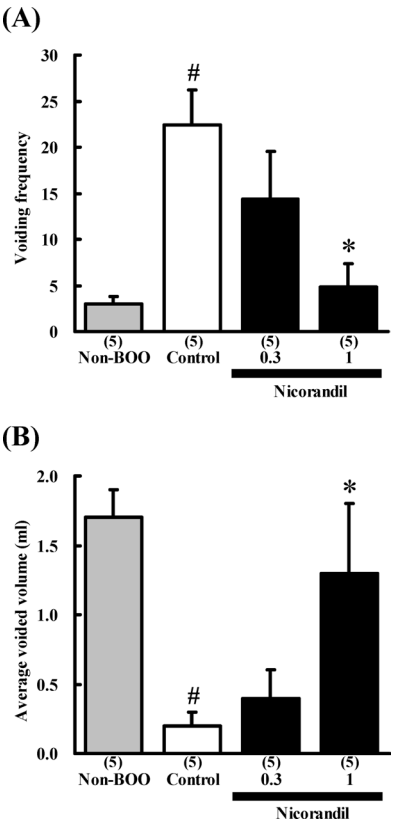

Fig. 1. Effects of Nicorandil on Voiding Behavior in Rats with BOO

(A) Voiding frequency, (B) average voided volume. Nicorandil (0.3—1 mg/kg) or vehicle was administered orally and each characteristic of voiding behavior was measured. Numbers in parentheses indicate the number of animals in each group. Each bar represents the mean±S.E.M. of 5 rats. Statistical significance was evaluated by Dunnett's test (*$p<0.05$). #$p<0.05$ between the non-BOO rats treated with vehicle (Non-BOO) and the BOO rats treated with vehicle (control) by Student's *t* test.

Nicorandil decreased voiding frequency and increased the voided volume significantly at the dose of 1 mg/kg (Fig. 1). In addition, nicorandil also significantly decreased the residual urine volume compared with the control group (Table 1). Cromakalim significantly decreased voiding frequency at 0.1 mg/kg (Fig. 2A). At the effective dose on voiding frequency, it had an inclination to increase in voiding volume per void and residual urine volume (Fig. 2B, Table 1). ISDN (1000 mg/kg) showed a significant decrease in voiding frequency and tended to decrease residual urine volume (Fig. 2A, Table 1). ISDN showed the tendency of increasing voiding volume per void (Fig. 2B).

The influence of nicorandil, cromakalim and ISDN on blood pressure and heart rate is shown in Table 2. Nicorandil did not affect blood pressure and heart rate at 1 mg/kg that improved frequent urination in BOO rats. On the other hand, cromakalim and ISDN decreased blood pressure and increased heart rate at the doses of 0.1 mg/kg and 1000 mg/kg that improved frequent urination in BOO rats, respectively.

DISCUSSION

In the present study, oral administration of nicorandil significantly decreased in voiding frequency and residual urine volume, and increased in voided volume per void at the dose which nicorandil didn't affect blood pressure. On the other hand, both cromakalim and ISDN decreased voiding fre-

quency at the dose which both drug decreased blood pressure. The precise mechanisms in which nicorandil had bladder-selectivity compared with the vasculature in the BOO rats remain to be clear, although the same potassium channel: SUR2B/Kir6.2, which was preferentially activated by nicorandil,[12] is located in both bladder and vascular smooth muscle.[13] One possible explanation could be that hybrid compounds with KCO and NO donor activity might have a strong efficacy compared with pure KCO or NO donor. Nicorandil relaxed bladder smooth muscle by KCO activity, leading to increase in bladder capacity during the storage phase.[2,9] Nicorandil has been also known to relax the urethral smooth muscle by two independent mechanism, opening of K_{ATP} channel and NO.[14] Both mechanisms will be functionally synergistic in increasing in voided volume per void and decreasing in the residual urine volume, leading to decrease in voiding frequency in bladder outflow obstruction model. Another hybrid compound, KRN2391 was also reported to have a strong efficacy to reduce bladder overactivity.[9,15] Furthermore, nicorandil was known as coronary artery-selective vascular relaxant so that it did not change in blood pressure in usual effective dose.[8,16] The same dose of nicorandil (1 mg/kg, *p.o.*) as in the present experiment did not affect blood pressure in conscious normal non-fasted rats, although the higher dose of oral nicorandil caused mild hypotension with a peak at 30 min after administration (unpublished observation).

Although cromakalim has been shown to inhibit bladder overactivity in rats, blood pressure was reduced at the same dose.[6] The clinical use of the first-generation KCO like cromakalim is dampened by the lack of selectivity for the bladder muscle. Indeed, cromakalim improved OAB patients in a pilot clinical study but the development of cromakalim as a treatment for OAB failed because of side effects such as hypotension.[17] Recently, based on the structure–activity relationship studies, several second-generation KCO, including ZD0947, ZD6169 and its derivative, ZM226600, WAY-133537 and A-251179 have been synthesized and their selective properties for the bladder have been reported from preclinical studies.[2,18—20] They were found to ameliorate bladder overactivity at a low dose to avoid inducing hypotension. Thus these agents may provide one of the basis for future treatment of frequent urination caused by OAB or BPH.

NO is an important regulator of the lower urinary tract function as well as the vascular tension. Neuroanatomic studies have demonstrated a rich nitrergic innervation and NOS enzyme activity in the urethra but sparse innervation in the detrusor in rats.[2,21,22] Exogenously applied NO-donors have been shown to relax pre-contracted urethral smooth mus-

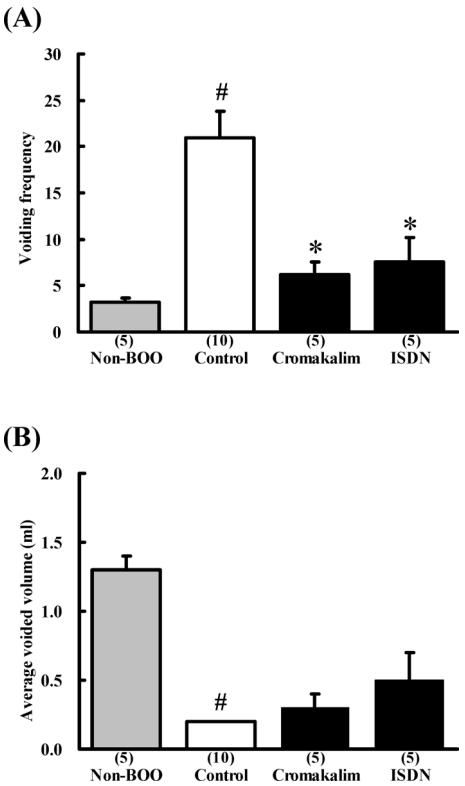

Fig. 2. Effects of Cromakalim and ISDN on Voiding Behavior in Rats with BOO

(A) Voiding frequency, (B) average voided volume. Cromakalim (0.1 mg/kg), ISDN (1000 mg/kg) or vehicle was administered orally and each parameter of voiding behavior was measured. Numbers in parentheses indicate the number of animals in each group. Each bar represents the mean±S.E.M. of 5—10 rats. Statistical significance was evaluated by Dunnett's test (*$p<0.05$). #$p<0.05$ between the non-BOO rats treated with vehicle (Non-BOO) and the BOO rats treated with vehicle (control) by Student's *t* test.

Table 1. Effects of Nicorandil, Cromakalim and ISDN on Residual Urine Volume in Rats

Treatment	Dose (mg/kg)	Number	Residual urine volume (ml)
Non-BOO[a)]	—	5	1.2±0.4
Control[a)]	—	5	4.0±0.5#
Nicorandil	0.3	5	2.8±0.5
Nicorandil	1	5	2.2±0.2*
Non-BOO[b)]	—	5	0.9±0.2
Control[b)]	—	10	4.0±0.6#
Cromakalim	0.1	5	3.9±0.8
ISDN	1000	5	2.4±0.5

a) Distilled water was administered orally as the corresponding vehicle for nicorandil. *b*) Three percent gum arabic solution was administered orally as the corresponding vehicle for cromakalim and ISDN. Data represent mean±S.E.M. from 5—10 rats. #$p<0.05$ between the non-BOO rats treated with vehicle (Non-BOO) and the BOO rats treated with the corresponding vehicle (control) by Student's *t* test. *$p<0.05$ between control and treatment group by Dunnett's test.

Table 2. Effects of Nicorandil, Cromakalim and ISDN on Blood Pressure and Heart Rate in BOO Rats

Treatment	Dose (mg/kg)	Number	Systolic blood pressure (mmHg)		Heart rate (beat/min)	
			Before	After	Before	After
Vehicle	—	5	150±4	154±4	411±22	412±32
Nicorandil	1	5	158±4	154±5	407±13	437±10
Cromakalim	0.1	5	147±5	118±5*	423±15	519±9*
ISDN	1000	5	157±4	126±3*	419±18	513±15*

Data represent mean±S.E.M. from 5 rats. Systolic blood pressure and heart rate were measured before and 30 min after oral drug administration. *$p<0.05$ by paired *t* test.

cle,[23] leading to decrease in intra-urethral pressure.[15] Sublingual administration of ISDN to humans was shown to decrease in the bladder outlet resistance.[24,25] Moreover, it was reported that endogenous and exogenous NO had inhibitory effects on the sensory afferent nerve activity in the bladder when the bladder was irritated by treatment with cyclophosphamide[7] or capsaicin in rat.[26] These studies support that NO plays physiological and pathophysiological roles in not only the relaxation of the urethra and urethral sphincter during micturition leading to decrease in urinary resistance during the emptying phase, but the suppression of afferent nerve function in the bladder, so that a bladder selective NO-donor without cardiovascular effects may be one of possible targets for medical treatment of LUTS.

Further experiments will be required to clarify the precise mechanism of bladder selectivity of nicorandil on frequent urination. The hybrid property of nicorandil, opening K_{ATP} channels and donating NO, seems to have advantages toward bladder selectivity over pure KCO or pure NO donor, which may provide another basis for future treatment of the frequent urination caused by OAB or BPH.

In conclusion, the present *in vivo* results showed that a hybrid of a K_{ATP} channel opener and NO donor, nicorandil ameliorated the frequent urination without decreasing blood pressure in BOO rats.

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