Inhibition by Benidipine of Contractility of Isolated Proximal and Distal Caprine Ureter

Abstract

Context: Benidipine is a calcium channel blocker that blocks all the major types (L, N, and T) of calcium channels. It has been shown to inhibit the contractility of many isolated smooth muscles but not isolated ureter. Aims: This study evaluated the ability of benidipine to inhibit the spontaneous contractility of isolated proximal and distal caprine (goat) ureter. Settings and Design: Spontaneous contractility of isolated goat ureter was recorded using a physiograph. Materials and Methods: Benidipine at concentrations in the range of 1 nM to 10 µM was analyzed for its inhibitory effects on the spontaneous contractility of the isolated proximal and distal caprine ureter. Statistical Analysis Used: Both parametric and nonparametric statistical tests were used. Results: The EC₅₀ of benidipine for inhibiting contractility in the distal ureter was found to be 54.68 nM. Benidipine was found to have a greater inhibitory effect on the distal ureter than on the proximal ureter. It was also found to inhibit amplitude of spontaneous ureteric contractility more readily than the frequency of spontaneous ureteric contractility. Conclusions: These results suggest that benidipine has differential inhibitory effects on the spontaneous contractility of the isolated ureter. Benidipine could be useful in the management of clinical conditions like ureteric colic due to its inhibitory effects on the contractility of the ureter.

Keywords: Benidipine, calcium channel blocker, contractility, ureter

Introduction

The function of the ureters is to transport urine from the kidneys to the urinary bladder. Normally, ureteric peristalsis starts with electrical activity at pacemaker sites found in the proximal part of the urinary collecting system.[1] The electrical activity then propagates distally and leads to peristaltic ureteric contractility which propels the bolus of urine distally.[1] Both the major autonomic pathways (adrenergic and cholinergic) regulate ureteric contraction.[2] Several neurotransmitters such as acetylcholine, noradrenaline, and histamine are involved in regulating ureteric contractility.[3] Various proteins such as muscarinic cholinergic receptors, alpha and beta adrenergic receptors, calcium channels, and potassium channels are involved in this process.[2,3] Calcium channels are known to play an important role in the contractility of the ureter.[4] Among the three major types of calcium channels (L-, N-, and T), it is thought that in the ureter, it is the L-type and not the N- and T-type, that are involved in regulating ureteric contractility.[4,5] Benidipine is a potent dihydropyridine calcium channel blocker (CCB) developed in Japan which has been approved for use in Japan and other Southeastern Asian countries for the treatment of hypertension and angina pectoris.[7] It is thought to block L-, N-, and T-type of calcium channels.[7] In addition to blocking calcium channels, it is also thought to exert other effects like stimulation of nitric oxide production, and it has antioxidant effects.[7] It can be administered orally once a day and has a good safety profile.[7] It has been shown to inhibit the contractility of various isolated smooth muscles such as rabbit mesenteric artery,[8] canine coronary artery,[9] and canine cerebral artery.[10] This paper reports the inhibitory effect of benidipine on the spontaneous contractility of isolated proximal and distal caprine (goat) ureter.

Materials and Methods

Tissue preparation

Ureters of freshly slaughtered 3–6-month-old goats with the urinary bladder were obtained from a local abattoir and transported to the laboratory in freshly prepared saline. After removal of adhering tissues, the ureters were cut into 2-cm-long segments, and the segments were used for experiments within 3 hours of excision. The segments were then attached to a 100-mL organ bath containing an oxygenated Krebs solution (118 mM NaCl, 4.7 mM KCl, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 11.5 mM glucose) at the desired length. The distal ends were tied to a fine wire, while the proximal ends were fixed to a force transducer. The resting tension was adjusted to 1 g, and the segments were allowed to stabilize for at least 1 hour.

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prepared mammalian Ringer solution. The composition of this solution was in mM: NaCl: 154; KCl: 5.6; CaCl$_2$: 2.2; NaHCO$_3$: 0.595; and dextrose: 5.55. The goat ureter was chosen for the study because of easy availability and anatomical, physiological, and histological similarities to the human ureter.$^{[11]}$ The distal 7 cm and proximal 4 cm of the ureter were used for the study. They were divided into strips measuring 1.5–1.8 cm long and the serosa was entirely removed. The ureteral lumen was opened using a blade, and then, the strips were mounted in an organ bath containing mammalian Ringer solution adequately aerated with oxygen and maintained at a temperature of 40°C. The study was approved by the Institutional Animal Ethics Committee and Review Board (IRB Min. No. 7785 dated March 9, 2012).

**Drugs**

Benidipine hydrochloride (Santa Cruz Biotechnology, Dallas, TX, USA) was dissolved in 15% methanol to give a 343.3 µM stock solution and stored at −20°C. All the other chemicals (NaCl, KCl, CaCl$_2$, NaHCO$_3$, and dextrose) used in the study were obtained from Qualigens, Mumbai, India.

**Experimental procedure**

Preliminary experiments were performed on the distal ureter to identify the concentrations of benidipine that elicited a minimal appreciable response and the maximal response by logarithmic increments in doses. The concentrations of benidipine chosen for the study were 60 nM, 300 nM, and 10 µM for proximal and distal ureters and 1 nM, 10 nM, 1 µM, and 3 µM for distal ureters. Tissues that failed to contract within this period were excluded from the study. A maximum acclimatization period of 10 min from the onset of spontaneous contraction was given for the tissues to stabilize, to avoid a spontaneous decline in tissue responses (observed in the control tissues) after 40 min from the onset of spontaneous contractions. To determine the dose-response relationship, a minimum of eight ureter specimens were assessed for each concentration of the drug. Thus, a total of 65 distal ureter specimens and 34 proximal ureter specimens were used. The control comprised the vehicle used for benidipine at its highest concentration, i.e., 15% methanol in 0.5 ml of double-distilled water.

**Analysis of data**

Contractility of the ureter was quantified by computing the tissue activity score$^{[12]}$ which is a product of frequency and amplitude over a period of 5 min and is considered to be the sum total of the tissue activity during this period. In comparison with analyzing frequency and amplitude of contractility separately, the tissue activity score could explain the overall effect of a drug on tissue activity. Statistical tests were performed to analyze tissue responses before and after administration of benidipine. Responses after administration of benidipine were also compared with responses after administration of the vehicle (15% methanol in 0.5 ml of double-distilled water). Dose-response analysis was performed by calculating the percent inhibition of tissue activity score of the last period (25–30 min after administration of the drug). The effects of benidipine on the frequency and amplitude of contractions were also determined.

Repeated measures ANOVA was used to assess the log-transformed tissue activity score over a period of 35 min, from 5 min before, the administration of drug, using the software R version: 3.2.2, R Foundation for Statistical Computing, Vienna, Austria. Post hoc comparisons were performed to determine the difference in activity score of the first and last time points for each set of specimens in the control and test arms. Bonferroni and Benjamini and Hochberg tests were used for post hoc analysis. Dose-response analysis was performed using the dose-response curve (DRC) package in software R. Four and five parameter log-logistic model and the Weibull model were compared to fit the curve to the data and to determine the EC$_{50}$ for the percentage inhibition of activity after 25 min of contact time with benidipine (activity between 25 and 30 min of contact with benidipine). Shapiro–Wilk test and lack of fit test were performed to determine the normality of data and to compare ANOVA with the DRC model, respectively.

**Results**

Spontaneous contractility was recorded over a period of 35 min. The latency to onset of spontaneous contractility varied from 2 to 90 min. Table 1 shows the effect of different concentrations of benidipine on tissue activity. As shown, benidipine inhibited the distal ureter more readily than the proximal ureter. At 10 µM benidipine concentration, in vitro inhibition of tissue activity was 81.08% and 100% for proximal and distal ureters, respectively. Post hoc analysis showed a significant reduction in the tissue activity score at 300 nM, 1 µM and 3 µM, and 10 µM concentrations of benidipine. Table 2 shows the inhibitory effect of benidipine on the frequency and amplitude of spontaneous contractility of the distal and proximal ureter at the 25–30 min time interval. As shown, for both frequency and amplitude of spontaneous ureteric contractility, benidipine had a greater inhibitory effect in the distal ureter than in the proximal ureter. The greater inhibition in the distal ureter compared to the proximal ureter was statistically significant for the frequency of spontaneous contractility at 10 µM benidipine concentration and for the amplitude of spontaneous contractility at 60 nM, 300 nM, and 10 µM benidipine concentrations [Table 2]. The EC$_{50}$ of benidipine was 54.68 nM for the distal ureter. Figure 1 shows the DRC for the inhibitory effect of benidipine on the proximal and distal ureter. Figure 2 shows the relationship between percentage inhibition of amplitude and frequency of contractility by benidipine over a 30 min contact period with different concentrations of benidipine used. As illustrated by the regression plane,
Benidipine inhibited the amplitude more than the frequency of spontaneous ureteric contractility and the differential inhibitory effect on amplitude and frequency of tissue activity was more marked in the proximal ureter. Figure 3 shows sample tracings of tissue activity of the isolated distal ureter at 25–30 min with and without the addition of 1 µM benidipine.

**Discussion**

This study has shown, for the first time, the dose-response relationship of the CCB benidipine on the spontaneous contractility of isolated proximal and distal caprine ureter. Benidipine reduced the tissue activity score, whereas the vehicle did not [Table 1 and Figure 3], indicating that benidipine inhibits the contractility of the isolated ureter. These results support previous studies conducted on the isolated ureter that have shown that CCB such as diltiazem, nifedipine, verapamil, and nicardipine inhibit ureteric contractility. All these CCB (diltiazem, nifedipine, verapamil, and nicardipine) block only the L-type calcium channel, unlike benidipine which blocks L-, N-, and T-type calcium channels. However, in the ureter, among the calcium channels, only the L-type calcium channels are thought to be present and involved in ureteric contractility. Hence, the inhibitory effect of benidipine on ureteric contractility found in the present study is likely to be due to the blockade of L-type calcium channels in the ureter.

In the present study, it was found that benidipine inhibited the contractility of the distal ureter at lower concentrations compared to the concentrations that inhibited the contractility of the proximal ureter [Tables 1 and 2]. The reason for this finding could be the presence of a greater number of L-type calcium channels in the lower ureter than in the upper ureter. It is interesting that similar results were obtained in a study on the relaxant effect of nifedipine on the isolated human ureter, where nifedipine had a greater relaxant effect in the distal ureter than on the proximal ureter. We also observed a differential inhibitory effect on the frequency and amplitude of spontaneous contractions in the presence of benidipine: the amplitude of ureteric contractions was inhibited more readily than the frequency of ureteric contractions [Table 2 and Figure 2]. The tissue

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**Table 1: Inhibitory effect of benidipine on tissue activity score**

| Concentration of benidipine | Distal | Proximal | Post hoc analysis (Greenhouse–Geisser correction) | Distal | Proximal | Post hoc analysis (Greenhouse–Geisser correction) |
|-----------------------------|--------|----------|-----------------------------------------------|--------|----------|-----------------------------------------------|
| Control                     | 0.74   | >0.05    | Mauchly’s test for sphericity not violated    | 0.77   | >0.05    | 0.42                                          |
| 1 nM                        | 3.56   | <0.05    | 0.07                                          | -      | -        | -                                             |
| 10 nM                       | 3.3    | <0.05    | 0.07                                          | -      | -        | -                                             |
| 60 nM                       | 4.8    | <0.05    | 0.052                                         | 2.57   | <0.05    | 0.14                                          |
| 300 nM                      | 7.98   | <0.05    | <0.05                                         | 3.1    | <0.05    | 0.08                                          |
| 1 µM                        | 16.19  | <0.05    | <0.05                                         | -      | -        | -                                             |
| 3 µM                        | 12.72  | <0.05    | <0.05                                         | -      | -        | -                                             |
| 10 µM                       | 21.51  | <0.05    | <0.05                                         | 5.65   | <0.05    | <0.05                                         |

**Table 2: Inhibitory effect of benidipine on frequency and amplitude of ureteric contractility at the 25 to 30 min time interval**

| Concentration of benidipine | Percentage of inhibition of frequency, mean (SEM) | P<sup>*</sup> | Percentage inhibition of amplitude, mean (SEM) | P<sup>*</sup> |
|-----------------------------|--------------------------------------------------|---------------|-----------------------------------------------|---------------|
|                             | Distal                                           | Proximal      | Distal                                        | Proximal      |
| Control                     | 21.08 (18.04)                                    | −21.66 (9.36) | 0.04                                          | −2.61 (14.77) | −12.70 (22.54) | 0.97 |
| 1 nM                        | −6.81 (10.35)                                    | −              | 0.29                                          | −14.78 (7.67) | −              |      |
| 10 nM                       | −12.57 (16.26)                                   | −              | 0.29                                          | 31.66 (7.64)  | −              |      |
| 60 nM                       | 30.59 (18.95)                                    | 2.98 (5.07)   | 0.37                                          | 53.28 (13.05) | −3.68 (17.09) | 0.02 |
| 300 nM                      | 70.05 (16.61)                                    | 34.5 (8.43)   | 0.37                                          | 57.17 (18.04) | −26.47 (29.93) | 0.01 |
| 1 µM                        | 67.41 (13.45)                                    | −              | 0.29                                          | 78.15 (26.51) | −              |      |
| 3 µM                        | 79.86 (12.93)                                    | −              | 0.29                                          | 86.19 (6.79)  | −              |      |
| 10 µM                       | 100 (0.00)                                       | 32.36 (26.58) | 0.03                                          | 100 (0.00)    | 85.52 (7.20)  | 0.03 |

SEM: Standard error of mean; *P value <0.05 was considered significant
distribution of calcium channels in the proximal and distal ureter needs to be determined to understand the role of CCB like benidipine on inhibition of ureteric contractility. To the best of our knowledge, this has not been done to date.

Ureteric colic is a common and important medical emergency commonly due to calculi. Urolithiasis (the presence of stones in the urinary tract) is a common clinical problem, with the lifetime risk estimated to be about 13% in men and 7% in women. One form of therapy that is used to treat ureteric colic is medical expulsion therapy (MET), the use of drugs to decrease the associated pain and to expel the calculus. Drugs used for MET include nonsteroidal anti-inflammatory drugs, CCB, and alpha adrenergic receptor blockers. A recent multi-center, randomized, placebo-controlled trial of an alpha adrenergic receptor blocker (400 µg tamsulosin) and a CCB (30 mg nifedipine) found that these drugs were not effective in decreasing the need for further treatment to achieve stone clearance in 4 weeks. The authors of this trial hence suggested that other drugs must be investigated for the treatment of ureteric colic due to stones. Since in the present study, benidipine was found to be more potent in inhibiting the contractility of the distal ureter than in the proximal ureter, benidipine could be a useful drug in MET since the differential inhibitory effects on the proximal and distal ureter could aid in expulsion of stones.

Hurtado et al. demonstrated the presence of T-type calcium channels along with hyperpolarization-activated cation channels in the pelvis-kidney junction, and showed that these channels play a crucial role in the origin of spontaneous peristaltic activity of the ureter which is transmitted distally along the ureteric smooth muscle cells through gap junctions. However, it must be noted that T-type calcium channels are thought to be present only in the pelvis-kidney junction, and not in the ureter. Since benidipine blocks L-, N-, and T-type calcium channels, and also approaches these channels through the cell membrane, it could confer prolonged inhibition of ureteric contractility and cause sustained pain relief in patients with ureteric colic.

**Conclusions**

This study has shown for the first time that benidipine has a differential inhibitory effect on the spontaneous contractility of the isolated proximal and distal caprine ureter. The predominant inhibitory effect of benidipine on ureteric contractility in the distal ureter compared to the proximal ureter may hasten the removal of calculi from the ureter.
the ureter. The results suggest that benidipine could be evaluated further for the treatment of ureteric stones.

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Conflicts of interest

There are no conflicts of interest.

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