Effects of β-Adrenergic Stimulation on the Acutely Obstructed Ureter in Dogs

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ABSTRACT

The objective of the present study was to evaluate the effects of a selective β3-adrenoceptor agonist, (R,R)-5-[2-[(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316243), on the acutely obstructed ureter in anesthetized dogs. After a complete ureteral obstruction produced by the inflation of a balloon catheter placed within the left lower ureter, the intraluminal ureteral pressure gradually rose to reach a plateau of 52.5 mm Hg. Intravenous administration of isoproterenol (a nonselective β-adrenoceptor agonist; 10 μg/kg) and CL 316243 (1 μg/kg) significantly decreased this elevated ureteral pressure (by 74.1 and 77.2%, respectively), with the reduction more sustained with CL 316243 than with isoproterenol. In addition, under both isoproterenol and CL 316243, urine flow (which had been interrupted by the balloon) was resumed, resulting in further sustained decreases in ureteral pressure. The mean blood pressure decreased and heart rate increased after the administration of both drugs, but these changes were greater in the isoproterenol group than in the CL 316243 group. In contrast, i.v. administration of butylscopolamine (an anticholinergic agent; 1000 μg/kg) had no evident effects on ureteral pressure or on urine flow. The increase in left kidney weight seen after ureteral obstruction was suppressed by CL 316243. We conclude that the selective β3-adrenoceptor agonist tested appears to be more useful than isoproterenol for reducing ureteral pressure above the obstructed site and for promoting ureteral relaxation and increasing urine flow around the point of obstruction in dogs.

Complete or partial ureteral obstruction can be caused by urinary calculi and can lead to edema, inflammation, and infection of the upper urinary tract, as well as to changes in ureteral functions (Biancani et al., 1976; Crowley et al., 1990). The resulting increase in intraluminal pressure in the upper urinary tract is the major cause of ureteral colic (Michaelson, 1974; Moriel et al., 1990). Previous clinical studies have shown that antispasmodics (e.g., anticholinergic agents, calcium antagonists, or papaverine) are effective for the relief of ureteral colic, and possibly for the promotion of stone passage (Ross et al., 1967; Jonsson et al., 1987; Borghi et al., 1994). However, a relaxant with more relative ureteral smooth muscle specificity compared with the cardiovascular system would result in potential clinical benefits.

The autonomic nervous system is known to play an important role in modulation of ureteral motility (Schulman, 1974; Morita et al., 1987). In the canine, facilitation of ureteral activity is mediated by the sympathetic nervous system, whereas inhibition is via the parasympathetic system (Del Tacca, 1978; Del Tacca and Taddei, 1977). The excitatory effects of cholinergic agonists are believed to result from both a direct muscarinic stimulation (Hernández et al., 1993) and an indirect release of catecholamines (Rose and Gillenwater, 1974). In the case of the sympathetic nervous system, adrenoceptors (ARs) and adrenergic nerves have been demonstrated in mammalian ureters (Latifpour et al., 1990; Edyvane et al., 1994), and in vitro and in vivo studies have both shown that α-AR agonists stimulate, whereas β-AR agonists inhibit, ureteral motility (Deane, 1967; Malin et al., 1970; Weiss et al., 1978; Morita et al., 1987). In the rabbit ureter, isoproterenol increases adenylate cyclase activity and the cAMP level, and these are reduced by propranolol (Weiss et al., 1977).

Recently, Tomiyama et al. (1998) indicated that there are significant species differences in the β-AR subtypes mediating the relaxation of ureteral smooth muscle: β1-ARs in the rat, β2-ARs in the rabbit, and β3-ARs in the dog. In the case of the isolated dog ureter, (R,R)-5-[2-[(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316243, a β3-AR agonist) and isoproterenol

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ABBREVIATIONS: AR, adrenoceptor; BP, blood pressure; HR, heart rate; LKW, left kidney weight; MBP, mean blood pressure; RKW, right kidney weight; UF, urine flow before ureteral obstruction; UFo, urine outflow; UP, ureteral pressure.
were both effective at relaxing the KCl-induced contraction than was either dobutamine (a β₁-AR agonist) or procaterol (a β₂-AR agonist). Park et al. (1997) showed the coexistence of β₃-ARs with β₂-ARs in the human ureter; CGP-12177 (a β₂-AR agonist), procaterol, and isoproterenol were more effective at relaxing either the spontaneous rhythmic contraction or the KCl-induced contraction than was dobutamine in the isolated human ureter. In addition, like in the human ureter, β₃-ARs are present in the human detrusor, and the relaxation induced by adrenergic stimulation is mediated mainly by β₂-ARs (Igawa et al., 1999).

In the present study, we attempted to evaluate the effects of a selective β₃-AR agonist on obstructive dysfunctions in the upper urinary tract using an acute ureteral obstruction model in the anesthetized dog. To this end, we compared the effects of isoproterenol (a nonselective β-AR agonist), CL 316243 (a β₃-AR agonist; Largis et al., 1994), and butylscopolamine (an anticholinergic agent) on the elevated intraluminal ureteral pressure (UP) and impeded urine flow in the obstructed ureter; we also compared the hemodynamic effects of these drugs. In addition, we observed obstructive changes in the kidney after the administration of isoproterenol, CL 316243, or butylscopolamine.

Materials and Methods

Animals. The animal experiments in this study were conducted in accordance with guidelines issued by the Kissei Pharmaceutical Co. Ltd. Animal Care and Use Committee. Adult beagle dogs (Nihon Nosen Kogyo, Yokohama, Japan) were housed individually with free access to tap water and commercial food pellets (CD-5; Nihon Clea, Osaka, Japan). The room temperature and humidity were ~23°C and ~55%, respectively, and a 12-h light/dark cycle was maintained.

Acute Ureteral Obstruction in Anesthetized Dogs. Dogs of either sex, weighing 8.0 to 14.0 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and immediately intubated. Artificial ventilation with room air was maintained using a volume-limited ventilator (SN-480-3; Shinano Seisakusyo, Tokyo, Japan). The dog was placed on a heating pad to minimize heat loss. Figure 1 shows a schematic representation of the experimental model. After an abdominal midline incision, the left ureter was identified. The left kidney was minimally dissected free from adjacent tissues, and a small nephromy was made on the convex side of the upper urinary tract using an acute ureteral obstruction model. To this end, we compared the methods of the acute ureteral obstruction model in the anesthetized dog.

Fig. 1. Schematic representation of the acute ureteral obstruction model in the anesthetized dog.

Experimental Protocol. After a rest period (~120 min) to allow stabilization of UP, UF, and hemodynamic parameters, the balloon was gradually inflated with water using an infusion pump (KN-201; Natsume Seisakusyo, Tokyo, Japan). Complete ureteral obstruction was considered to have been achieved when UP stopped. The volume of water infused into the balloon was stable in each dog (~20 μl). When UP had risen and barely changed for more than 10 min, it was considered to be the plateau UP, and vehicle or one dose of one drug was injected i.v. The dogs were divided into seven groups depending on whether they received physiological saline (vehicle, n = 6); isoproterenol at 1 μg/kg (n = 4) or 10 μg/kg (n = 5); CL 316243 at 0.1 μg/kg (n = 4); or 1 μg/kg (n = 4); or butylscopolamine at 1000 μg/kg (n = 4). UP, BP, and HR were all recorded on a thermowriting rectigraph (WS-681G; Nihon Kohden). Throughout the experiment, physiological saline (10 ml/kg/h) was infused intravenously to ensure a stable urine flow. Anesthesia was maintained with an infusion of sodium pentobarbital (3-5 mg/kg/h i.v.).

Histological Determination. Dogs were sacrificed with an overdose of sodium pentobarbital 120 min after drug administration. The left and right kidneys were immediately excised and weighed and then immersed in buffered 10% formalin. The kidneys were cut into sections 3 to 4 μm thick. The sections were stained with H&E. Histopathological findings were classified into four grades: no remarkable change (0), slight change (1), moderate change (2), and severe change (3). The grading of histopathological findings was performed by an observer who was blind to the treatment group from which the tissue was obtained. Both the rate and the degree of tubules with dilated lumen and flatted epithelium existing in a section were completely judged.

Drugs. Isoproterenol [(−)-isoproterenol bitartrate] and butylscopolamine [(−)-scopolamine N-butyl bromide] were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium pentobarbital was obtained from Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan). Sodium heparin was obtained from Marion Merrell Dow Co. Ltd.
(Tokyo, Japan). CL 316243 was synthesized by Kissei Pharmaceutical Co. Ltd. (Hotaka, Japan). All drugs were dissolved in physiological saline.

Statistical Analysis. The data are expressed as mean ± S.E. A one-way ANOVA was used for the statistical analysis of multiple comparisons within each group. When a significant difference was detected by the one-way ANOVA, the data were further analyzed with Dunnett’s test. A Student’s t-test for unpaired data was used when comparisons were made between two groups. The difference between UFo values was analyzed with Wilcoxon’s sign rank test because the data were not normally distributed. A value of P < .05 was considered to indicate statistical significance.

Results

Effects of Isoproterenol, CL 316243, and Butylscopolamine on UP, BP, and HR. Before ureteral obstruction, rhythmic UP waves associated with each ureteral peristalsis were observed in anesthetized dogs. In terms of the baseline UP, mean blood pressure (MBP), and HR, there were no significant differences among the vehicle-, isoproterenol (1 and 10 μg/kg i.v.), CL 316243 (0.1, 0.3, and 1 μg/kg i.v.), and butylscopolamine (1000 μg/kg i.v.)-treated groups (Table 1). Within 5 min of complete ureteral obstruction, both the peristaltic rate and the peak UP increased transiently (Fig. 2). The baseline UP then reached a plateau, and the ureteral peristalsis disappeared. The plateau UP was 52.5 ± 6.9 mm Hg (n = 6) and the time required to reach the plateau after ureteral obstruction was 86.2 ± 4.8 min (n = 6) in vehicle-treated dogs. Neither the UP, MBP, and HR values recorded before drug administration nor the time between ureteral obstruction and drug administration was significantly different among the seven treatment groups (Table 1). In the vehicle-treated group, UP, like MBP and HR, barely changed after 120 min after its administration (see Figs. 4–6).

Figure 3 shows typical recordings of the effects of isoproterenol (10 μg/kg i.v.; Fig. 3A), CL 316243 (1 μg/kg i.v.; Fig. 3B), and butylscopolamine (1000 μg/kg i.v.; Fig. 3C) on UP, BP, and HR. Intravenous administration of isoproterenol at dosages of 0.1, 0.3, and 1 mg/kg induced a dose-dependent decrease in MBP and increase in HR (Fig. 5). In 1 mg/kg CL 316243-treated dogs, UP was decreased by 54.3 ± 6.5% (n = 4) at 20 min; it then gradually recovered, only to fall again from 60 to 120 min. In 1 mg/kg CL 316243-treated dogs, UP was decreased by 77.2 ± 6.1% (n = 4) at 20 min, and this decrease was sustained for an additional 100 min; as a result, UP stayed between 8.2 ± 4.0 mm Hg (n = 4) and 13.0 ± 3.0 mm Hg (n = 4) from 20 to 120 min. CL 316243 at doses of 0.3 and 1 mg/kg induced a gradual decrease in MBP and increase in HR (Fig. 5). The maximum effects were achieved with 1 mg/kg at 30 min, with MBP being decreased by 12.7 ± 4.0% (n = 4) and HR increased by 28.2 ± 5.1% (n = 4) at this time.

Butylscopolamine at a dose of 1000 μg/kg transiently decreased UP within 10 min of administration in three of the four animals (by 13.5, 36.4, and 42.7% in each dog) but increased UP in a remaining animal (by 20.9%), failing to show statistically significant effects on UP (Fig. 6). Both MBP and HR were decreased by 29.6 ± 3.4% (n = 4) and 18.3 ± 2.8% (n = 4) at 2 min, respectively (Fig. 6). Atrioventricular block was transiently observed in two of the four animals within 5 min of administration.

Effects of Isoproterenol, CL 316243, and Butylscopolamine on UFo. Figure 7 shows the effects of isoproterenol, CL 316243, and butylscopolamine on UFo from the obstructed ureter in anesthetized dogs. Before ureteral obstruction, UF values were not significantly different among the vehicle-, isoproterenol (1 and 10 μg/kg i.v.), CL 316243 (0.1, 0.3, and 1 μg/kg i.v.), and butylscopolamine (1000 μg/kg i.v.)-treated groups. After ureteral obstruction, UFo was not observed for 120 min in vehicle-treated dogs (with the exception of a small UFo from 80 to 120 min in one of the six

### Table 1

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose μg/kg i.v.</th>
<th>n</th>
<th>UP Obstruction (mm Hg)</th>
<th>MBP Administration μm Hg</th>
<th>HR Beats/min</th>
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<tr>
<td>Vehicle</td>
<td></td>
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<td>51.5 ± 9.2</td>
<td>147.8 ± 7.8</td>
<td>163 ± 16</td>
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<td>Isoproterenol</td>
<td>1</td>
<td>4</td>
<td>3.6 ± 12.1</td>
<td>54.6 ± 5.6</td>
<td>159 ± 16</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>3.5 ± 6.6</td>
<td>57.1 ± 6.2</td>
<td>152 ± 9.8</td>
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<tr>
<td></td>
<td>CL 316243</td>
<td>0.1</td>
<td>4.2 ± 1.8</td>
<td>54.1 ± 3.3</td>
<td>142.5 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>4</td>
<td>5.2 ± 2.1</td>
<td>65.9 ± 8.2</td>
<td>152.9 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>1.6 ± 0.8</td>
<td>57.6 ± 5.1</td>
<td>145.0 ± 3.8</td>
</tr>
<tr>
<td>Butylscopolamine</td>
<td>1000</td>
<td>4</td>
<td>0.6 ± 0.4</td>
<td>60.5 ± 8.9</td>
<td>153.8 ± 6.3</td>
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</table>

*P < .05 versus before ureteral obstruction.
We also measured the urine flow from the right (nonobstructed) ureter in the same dogs; it did not change significantly for 120 min (data not shown).

In almost all dogs in the isoproterenol- and CL 316243-treated groups, the first trace of UFo was observed during the recovery from the maximum reduction in UP induced by the drugs, and a continuous UFo developed thereafter, whereas the distribution was rather skewed in each dog. Isoproterenol at a dose of 10 μg/kg significantly increased UFo from 40 to 120 min; UFo was present in each of the five animals from 80 to 120 min. UFo was increased from 40 to 80 min by 0.3 μg/kg CL 316243 and from 40 to 120 min by 1 μg/kg CL 316243. UFo was present in each of the four animals from 60 to 120 min in 1 μg/kg CL 316243-treated dogs. In contrast, butylscopolamine (1000 μg/kg) did not increase UFo significantly. UFo was not observed for 120 min in two of the four animals.

**Effects of Isoproterenol, CL 316243, and Butylscopolamine on Kidney.** Table 2 shows the left (obstructed) kidney weight (LKW), right (nonobstructed) kidney weight (RKW), and the LKW/RKW ratio 120 min after the administration of vehicle, isoproterenol (1 and 10 μg/kg i.v.), CL 316243 (0.1, 0.3, and 1 μg/kg i.v.), and butylscopolamine (1000 μg/kg i.v.). In addition, we weighed both kidneys in normal (without ureteral obstruction or drug administration) dogs. LKW/RKW was significantly greater in vehicle-treated dogs than in normal dogs. CL 316243 at doses of 0.3 and 1 μg/kg significantly and dose dependently suppressed the increase in LKW/RKW to an extent close to that in normal dogs and also tended to decrease LKW, indicating that CL 316243 attenuated the increase in LKW seen after ureteral obstruction.

Under the light microscope, a dilatation of renal tubules could be seen in the left kidney (Fig. 8B) but not in the right kidney (Fig. 8A) of vehicle-treated dogs. Although such changes were also observed in the obstructed kidneys in all dogs, we did not find severe (grade 3) dilatation of tubules in either 10 μg/kg isoproterenol- or 1 μg/kg CL 316243-treated dogs, but we did in butylscopolamine-treated dogs (Table 3, Fig. 8, C and D).
Discussion

Acute Ureteral Obstruction in Dogs. Acute ureteral obstruction increases the intraluminal pressure in the upper urinary tract, and the elevated UP is responsible for inducing changes in both ureteral functions (Biancani et al., 1976; Crowley et al., 1990) and renal hemodynamics (Moody et al., 1975). In the present study, we achieved acute ureteral obstruction in dogs through the inflation of an intraluminal balloon catheter to simulate the effects of an incarcerated calculus on ureteral functions; the technique we used involving minor modifications of previously reported methods (Stower et al., 1986; Crowley et al., 1990). UP rose gradually and reached a plateau at 52.5 mm Hg within 86.2 min of ureteral obstruction and then showed no decline for an additional 120 min in vehicle-treated dogs (Table 1, Figs. 4–6). This time course is consistent with previous observations in dogs with a complete ureteral obstruction (Darracott Vaughan et al., 1971; Moody et al., 1975). Thus, our model mimicked quite closely the clinical situation in which obstructive dysfunctions result from the presence of incarcerated calculi, including elevated intraluminal pressure and stagnated urine in the upper urinary tract. In addition to our measurements of UP, we observed a resumption in urine flow after its interruption by the balloon (UFo), obstructive changes in the kidney, and hemodynamic parameters. By using this model of acute ureteral obstruction in dogs, we could study the effects of β-adrenergic stimulation on ureteral, renal, and cardiovascular functions.

Effects on UP. Previous in vitro and in vivo studies have demonstrated that β-adrenergic stimulation inhibits ureteral motility. Isoproterenol produces relaxation of isolated ureters in a number of species (Malin et al., 1970; Weiss et al., 1978; Morita et al., 1987; Tomiyama et al., 1998), whereas it
Effects of isoproterenol, CL 316243, and butylscopolamine on LKW, RKW, and LKW/RKW

TABLE 2

Effects of isoproterenol, CL 316243, and butylscopolamine on LKW, RKW, and LKW/RKW

Values are mean ± S.E.

<table>
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<tr>
<th>Treatment Group</th>
<th>Dose</th>
<th>n</th>
<th>LKW</th>
<th>RKW</th>
<th>LKW/RKW</th>
</tr>
</thead>
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<td></td>
<td>μg/kg i.v.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.0</td>
<td>6</td>
<td>29.31 ± 2.22</td>
<td>23.20 ± 1.72</td>
<td>1.26 ± 0.01</td>
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<tr>
<td>Isoproterenol</td>
<td>1.0</td>
<td>4</td>
<td>27.91 ± 1.88</td>
<td>21.49 ± 1.33</td>
<td>1.35 ± 0.04</td>
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<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>25.81 ± 1.36</td>
<td>22.30 ± 0.96</td>
<td>1.16 ± 0.02</td>
</tr>
<tr>
<td>CL 316243</td>
<td>0.1</td>
<td>4</td>
<td>25.55 ± 0.81</td>
<td>20.60 ± 0.64</td>
<td>1.24 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>4</td>
<td>25.13 ± 1.95</td>
<td>23.59 ± 1.08</td>
<td>1.06 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4</td>
<td>23.80 ± 0.80</td>
<td>23.31 ± 0.97</td>
<td>1.02 ± 0.02*</td>
</tr>
<tr>
<td>Butylscopolamine</td>
<td>1000</td>
<td>4</td>
<td>27.08 ± 1.36</td>
<td>22.88 ± 1.54</td>
<td>1.19 ± 0.03</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>25.62 ± 0.99</td>
<td>23.79 ± 1.27</td>
<td>0.98 ± 0.02*</td>
</tr>
</tbody>
</table>

* P < .05 versus vehicle-treated group.

Normal dogs (without left ureteral obstruction or drug treatment).
obstructed site and the relaxation at the obstructed site itself. Furthermore, other effects of β-adrenergic stimulation could possibly modify UFo, such as a decrease in ureteral peristalsis or an inhibition of urine bolus formation (Mayo and Halbert, 1981; Morita et al., 1987). On the basis of these results, it is speculated that relaxation of ureteral muscle by β-adrenergic stimulation may promote urine flow around incarcerated calculi; by decreasing ureteral wall tension, there may be decreased force of coaptation between the point of obstruction and the ureteral wall, which may actually decrease the pressure gradient across the obstructed site. Such a resumption in urine flow would contribute to a sustained reduction in UP and could possibly promote stone passage, although further experimental studies, including the development of new ureteral obstruction models using an artificial calculus, are needed to clarify the effects of β-adrenergic stimulation on urine flow and stone passage. In addition, when taken together with the fact that UFo developed variably in each preparation after drug administration, we must consider the existence of uncontrolled/ uncontrollable renal and prerenal factors affecting UFo and UP (e.g., an extravasation of urine at calices, a hydration status, or an osmotic diuresis) in this model of acute ureteral obstruction in dogs.
Conclusion. We have demonstrated that the relaxation of ureteral smooth muscle by CL 316243, a selective β3-AR agonist, decreases the elevated UP and resumes urine flow in the acutely obstructed ureter in dogs. These findings are consistent with the idea that selective adrenergic stimulation of ureteral β3-ARs may prove to be useful for relieving ureteral colic and promoting stone passage in patients with urolithiasis. In the human ureter, the relaxation induced by adrenergic stimulation is mediated by both β2- and β3-ARs (Park et al., 1997). Because β3-ARs are widely distributed in the cardiovascular system, we believe that a specific agonist for human ureteral β3-ARs with comparatively weak hemodynamic effects would be extremely useful for the drug treatement of urolithiasis; additional acute and chronic studies are needed to confirm this concept.

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References


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