Effect of solifenacin with and without antioxidant supplements on the response to experimental outlet obstruction and overactive bladder dysfunction in rabbits: Part 1

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OBJECTIVE: One of the most common forms of urinary dysfunction is related to the development of overactive bladder (OAB) dysfunction. Solifenacin is a relatively new selective antimuscarinic agent that has been shown to be particularly useful in treating OAB dysfunction in both men and women. Experimentally, we have previously demonstrated that OAB is associated with the generation of free radicals and oxidative damage to the bladder. We tested the hypothesis that the combination of solifenacin + coenzyme Q10 (CoQ10) + α-lipoic acid (α-LA) is more effective in treating OAB than the individual compounds.

MATERIALS AND METHODS: In total, 48 male New Zealand White rabbits were separated into 8 groups of 6 rabbits each. The following oral treatments were given to each group: groups 1 and 5 received vehicle (saline); groups 2 and 6 received solifenacin; groups 3 and 7 received CoQ10 + α-LA; and groups 4 and 8 received solifenacin + CoQ10 + α-LA. After 3 weeks of treatment (by oral gavage), rabbits in groups 1 to 4 received partial outlet obstruction. The rabbits continued their treatments for 4 weeks following surgery. At the end of this 4-week period, each rabbit received cystometry and then underwent an in situ study for OAB.

RESULTS: The results clearly demonstrated that obstructive bladder dysfunction and the level of OAB were reduced in all three treatment groups, but the combination of solifenacin + antioxidants was significantly more effective than either solifenacin or antioxidants alone.

CONCLUSION: The addition of the antioxidants CoQ10 + α-LA worked synergistically with solifenacin in the treatment of obstructive bladder dysfunction and OAB.

1. Introduction

The urinary bladder is a smooth-muscle organ, which functions to collect and store urine at low intravesical pressures, and then to periodically expel the urine via highly coordinated sustained contractions. One of the most common forms of dysfunction is related to the development of overactive bladder (OAB) dysfunction. Solifenacin is a relatively new selective antimuscarinic agent that has been shown to be particularly useful in treating OAB dysfunction in both men and women. Experimentally, we have previously demonstrated that OAB is associated with the generation of free radicals and oxidative damage to the bladder. We tested the hypothesis that the combination of solifenacin + coenzyme Q10 (CoQ10) + α-lipoic acid (α-LA) is more effective in treating OAB than the individual compounds.

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distribution in the bladder, and thus modify solifenacin’s activity.12–14 The etiology of bladder dysfunction secondary to BPH (OAB and poor bladder contraction) is in part due to the generation of free radicals and oxidative damage to the bladder’s smooth muscles and nerves.5,10

Coenzyme Q10 (CoQ10) is a lipid-soluble cofactor found naturally in mitochondria and carries out important biochemical functions in mitochondrial inner membranes. CoQ10 serves as an electron and proton carrier for energy coupling and helps maintain an electrical gradient across cell membranes for ATP production.17,18 CoQ10 also serves as a primary scavenger for free radicals and an indirect stabilizer of calcium channels to decrease calcium overload.

α-Lipoic acid (α-LA) is also naturally found in mitochondria, and acts as a critical coenzyme for mitochondrial enzymes, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase.19 Its reduced form, dihydrolipoic acid, and other metabolites have strong antioxidant effects as reactive oxygen species scavengers, and act as chelators of such transition metals as magnesium (Mn⁺), zinc (Zn⁺) and copper (Cu).20,21 Supplementation with α-LA has also been shown to be an effective glutathione (GSH) substitute and to increase cellular GSH levels.22 The primary biological function of GSH is to act as a nonenzymatic reducing agent, preventing oxidative stress in most cells and helping to trap free radicals that can damage DNA and RNA.

The combination of CoQ10 and α-LA has proven to be very potent at preventing oxidative damage to the bladder caused by both direct ischemia/reperfusion and partial outlet obstruction.23–25 This combination of supplements significantly protects against the contractile, biochemical, and structural dysfunctions associated with increased free radical production.

The current study was designed to evaluate the hypothesis that solifenacin works in synergy with CoQ10 and α-LA in the treatment of obstructive bladder dysfunction and OAB dysfunction.

2. Materials and methods

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Stratton VA Medical Center, Albany, NY. Our current study used both an in vivo and a novel in situ model to study the effects of solifenacin with or without antioxidants on the direct response of the bladder to intra-arterial Ach, obstructive bladder dysfunction, and OAB dysfunction. These experiments were based upon published techniques.10–14

2.1. Cystometry

Each rabbit was anesthetized with 1–3% isoflurane by inhalation. The bladder was catheterized through the urethra with an 8-Fr Foley catheter and filled at 1 mL/minute warmed saline, while the intravesical bladder pressure was continually monitored (by cystometry). If OAB was present, cystometry was stopped, and the frequency and amplitude of the OAB were recorded, and then cystometry was completed.

2.2. Partial outlet obstruction

Each rabbit was anesthetized with isoflurane, and the bladder was catheterized with an 8-Fr Foley catheter via the urethra, and then exposed through a midline incision. A 00 silk ligature was snugly tied around the catheterized proximal urethra, and the catheter was removed.26–28 This method has been shown to be an excellent model of BPH-induced obstructive bladder dysfunction in men.29,30 Approximately 50% of these rabbits developed OAB as observed during cystometry. Those rabbits that showed OAB progressed to severe bladder dysfunction at a significantly faster rate than rabbits that did not show OAB. This result is similar to what occurs in human males with BPH and OAB. The etiology of obstructive bladder dysfunction is via ischemia/reperfusion and free radical generation and damage.

2.3. In situ OAB31–33

After 4 weeks of obstruction, each rabbit was anesthetized as described previously, and the right external carotid artery was cannulated for blood pressure monitoring. A polyethylene catheter was inserted through the rabbit’s right femoral artery until it reached the lower abdominal aorta. A heparinized saline-filled polyethylene catheter was used for intra-arterial administration of Ach. The bladder was exposed through a midline incision of the abdominal wall and it was catheterized through the bladder dome with an 8-Fr Foley catheter for both monitoring of the bladder pressure and infusion of saline. At this point, a ligature was placed around the external proximal penis, the bladder was filled with 30 mL warmed saline, and the bladder pressure was monitored. Ach was given intra-arterially, and the maximal pressure generated was recorded. Within 30 minutes, the majority of rabbits had developed OAB; and the amplitude and frequency of contractile activity were recorded.

Therefore, these models provided quantitative data on: (1) the micturition pressure; (2) the volume that initiated micturition; (3) compliance (taken from the cystometric curve); (4) the response to intra-arterial Ach; (5) the number of animals that developed overactivity; and (6) the amplitude and frequency of bladder overactivity.

2.4. Experimental design

Forty-eight male New Zealand White rabbits were separated into eight groups of six rabbits each. The following oral treatments were given: groups 1 and 5 received the vehicle (saline); groups 2 and 6 received solifenacin (0.3 mg/kg/day); groups 3 and 7 received 100 μM/kg/day α-LA; groups 4 and 8 received 0.3 mg/kg/day solifenacin + 100 μM/kg/day α-LA and 5 mg/kg/day CoQ10.

After 3 weeks of treatment (by oral gavage), rabbits in groups 1 to 4 were anesthetized with 1–3% isoflurane and received partial outlet obstruction as detailed above. The rabbits continued their treatments for 4 weeks following surgery.

Immediately following the in situ experiment, the bladder was excised and weighed, and three full-thickness bladder strips were taken for in vitro contractile studies. The remainder of the bladder was separated by blunt dissection into muscle and mucosal compartments, frozen under liquid nitrogen, and stored at −80°C for biochemical experiments.

2.5. In vitro contractile studies

Each isolated strip was suspended between platinum electrodes in an individual 15-mL bath containing Tyrode’s solution with 1 mg/mL glucose and equilibrated with 95% oxygen and 5% carbon dioxide at 37°C. After 30 minutes of equilibration, 2 g of tension were placed on each strip. This is the tension required to obtain maximal contractile responses to field stimulation (FS). After 15 minutes of equilibration at 2 g of tension, each strip was subjected to FS at 2 Hz, 8 Hz, and 32 Hz with 80 V and a 1-millisecond duration; the maximal response was recorded on a model D Grass polygraph; and the analog signal was digitized using the Polyview system. At 10 minutes after FS, each strip was stimulated by the addition of 10 μM carbachol, and the maximal contractile
response was recorded. Each strip was washed three times at 10-minute intervals with fresh, warmed, oxygenated Tyrode solution and then stimulated by the addition of 1 mM of ATP. After another series of washes, the strips were stimulated with 120 mM of potassium chloride (KCl), and the maximal response was recorded. We found that producing dose–response curves with these agents produced significant fatigue, which then made the data unreliable. Giving a single maximal dose did not cause fatigue.

Part 1 reports on the urodynamic and physiological results of this study and part 2 reports on the biochemical aspects of this study.

3. Results

Partial outlet obstruction caused a significant increase in bladder weight ($p < 0.05$) in all groups (Table 1). However, bladder weight in the obstructed antioxidant group and the obstructed solifenacin + antioxidant groups was significantly lower than that of the obstructed group with vehicle. Additionally, the bladder weight in the obstructed solifenacin + antioxidant group was significantly lower than that of the obstructed antioxidant group. In general, the lower the obstructed bladder weight, the greater the contractile and metabolic function.

Figure 1 displays the cystometric curves for the various groups. The obstructed group with vehicle had a steeper curve than any of the other groups, showing that the compliance of this group was low (poor) compared with the compliance of the treatment groups. Quantitative values for compliance are presented in Table 1. None of the treatments had any effect on the compliance of the control groups. The obstructed group with vehicle had a significantly lower (poorer) compliance than any of the other obstructed groups. All treatments resulted in the compliance returning to control values.

Obstruction resulted in significant and similar increases in all micturition pressures (Table 1). There were no significant differences among the groups in the volume at micturition primarily because of the large standard errors. The combination of solifenacin + antioxidants had the most consistent volume at micturition. The percentage of rabbits showing OAB dysfunction, and the amplitude and frequency of OAB are presented in Table 1. Since there were only six rabbits per group, no standard error of the mean could be calculated; however, it appeared that all treatments reduced the percentage of rabbits showing OAB in the control groups. Obstruction increased the percentage of rabbits showing OAB in all groups; however, this increased percentage in the antioxidant and solifenacin + antioxidants groups was lower than that compared with the other two groups. This finding was unexpected and may have important clinical implications.

There appeared to be a decrease in the amplitude of OAB in both control and obstructed rabbits associated with all treatments, especially in the solifenacin + antioxidants obstructed group. There also appeared to be a decrease in the frequency of OAB in the obstructed bladders of all treatment groups and in the control bladders of the solifenacin + antioxidant groups.

Figure 2 displays the in situ response to intra-arterial Ach. Obstruction caused significant decreases in the response to Ach in all groups. However, the Ach response of the obstructed group treated with the combination of solifenacin + antioxidants was significantly greater than that of the obstructed group with vehicle.

Figure 3 shows the contractile response to FS, carbachol, and KCl by control bladders. There were no effects of any of the treatments on the control responses (data not shown). Figure 4 shows the effect of partial outlet obstruction on the contractile responses to all forms of stimulation as a percentage of the control response. Contractile responses of the combined solifenacin + antioxidant obstructed group to all forms of stimulation were significantly increased compared with those in the obstructed group with vehicle. Contractile responses of the antioxidant obstructed group to all forms of stimulation, except for 2 Hz FS, were significantly increased compared with those in the obstructed group with vehicle. Contractile responses of the solifenacin obstructed group to

### Table 1

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>Obstructed</th>
<th>Solifenacin</th>
<th>CoQ + αLA</th>
<th>Solifenacin + CoQ + αLA</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Bladder weight (g)</td>
<td>2.2 ± 0.23</td>
<td>9.8 ± 4.14</td>
<td>2.6 ± 0.25</td>
<td>9.18 ± 0.69</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Compliance (cmH2O/20% capacity)</td>
<td>5.7 ± 0.46</td>
<td>16 ± 4.25</td>
<td>5.77 ± 0.46</td>
<td>5.9 ± 1.06</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>Micturition pressure (cmH2O)</td>
<td>21 ± 1.3</td>
<td>40 ± 6.04</td>
<td>19.3 ± 1.3</td>
<td>31.5 ± 6.04</td>
<td>24.8 ± 1.3</td>
</tr>
<tr>
<td>Volume at micturition (mL)</td>
<td>20 ± 7</td>
<td>30 ± 12</td>
<td>30 ± 10</td>
<td>30 ± 8</td>
<td>22 ± 10</td>
</tr>
<tr>
<td>Percentage of rabbits showing OAB</td>
<td>50 ± 7</td>
<td>66 ± 16</td>
<td>16 ± 66</td>
<td>16 ± 33</td>
<td>16 ± 33</td>
</tr>
<tr>
<td>Amplitude of OAB (cmH2O)</td>
<td>3.1 ± 1.1</td>
<td>6.2 ± 1.5</td>
<td>0.35 ± 0.11</td>
<td>3.6 ± 0.87</td>
<td>0.13 ± 0.2</td>
</tr>
<tr>
<td>Frequency of OAB (no. of contractions/10 min)</td>
<td>1.3 ± 0.3</td>
<td>5.5 ± 0.99</td>
<td>0.1 ± 0.11</td>
<td>3.0 ± 0.21</td>
<td>0.4 ± 0.11</td>
</tr>
<tr>
<td>Response to intra-arterial acetylcholine cmH2O/g</td>
<td>3.9 ± 0.07</td>
<td>1.5 ± 0.22</td>
<td>3.77 ± 0.2</td>
<td>1.4 ± 0.15</td>
<td>3.6 ± 0.5</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of six individual rabbits per group, except for the percentage of animals showing OAB values.

- $^a$ $p < 0.05$ compared to the control group for each treatment.
- $^b$ $p < 0.05$ compared to the obstructed group without group (vehicle).

CoQ = coenzyme Q10; αLA = α-lipoic acid; Ach = acetylcholine; OAB = overactive bladder.
carbachol and KCl were significantly increased compared with those in the obstructed group with vehicle.

4. Discussion

Antimuscarinic agents have proven to be clinically effective in treating OABs in both men and women.34–36 These drugs relax the bladder by muscarinic cholinergic inhibition, which makes them useful drugs for treating an unstable bladder. Since both bladder overactivity and micturition are based on neuronal release of Ach and muscarinic receptor stimulation, it is important to directly compare the effect of antimuscarinic agents on these two cholinergic systems in a controlled, quantitative manner, in which the true sensitivity can be determined for each system. Moreover, it would be beneficial to discover treatment modalities that address overactivity without affecting the bladder contractile force. This benefit might be accomplished through the use of antioxidants as mentioned in the Introduction section. For the current studies, we chose to use a combination of CoQ10 and α-LA because, as mentioned earlier, this combination of antioxidants has been shown to be most effective in treating partial outlet obstruction and in situ ischemia/reperfusion.23,24,37

CoQ10 is a lipid-soluble cofactor found naturally in mitochondria that carries out important biochemical functions in mitochondrial inner membranes. CoQ10 serves as an electron and proton carrier for energy coupling, and helps maintain an electrical gradient across cell membranes for ATP production.17,18 CoQ10 also serves as a primary scavenger of free radicals and an indirect stabilizer of calcium channels to decrease calcium overload. Similar to its effects in the bladder, preoperative oral CoQ10 therapy in patients undergoing cardiac surgery increases myocardial and cardiac mitochondrial CoQ10 levels, improves mitochondrial efficiency, and increases myocardial tolerance to in vitro hypoxia-reoxygenation stress.38

In addition to its effects on the urinary bladder, α-LA protects against hepatic ischemia/reperfusion injury and other forms of oxidative stress in rats.39,40

These studies used two models of bladder overactivity. The first is an in situ method that creates OAB in normal rabbits and was effectively used in studies on the effects of intravesical and intravaginal treatments of bladder overactivity.31,32,41,42 The second is a model of chronic partial bladder outlet obstruction. Approximately 50% of obstructed rabbits developed significant OAB. In both models, bladder damage was mediated in part by the generation of free radicals and oxidative damage to nerves, mitochondria, and the sarcoplasmic reticulum.15,16,43–45 Partial outlet obstruction results in significant changes in the bladder structure, including detrusor hypertrophy, mucosal hyperplasia, mucosal and detrusor angiogenesis, and significant collagen synthesis and deposition.29,46–51 These structural changes include significant damage to nerves, synapses, mitochondria, and the sarcoplasmic reticulum.29,46,48 This structural damage plays a role in the dysfunction observed in this model.

The following is a summary of our findings. The following parameters of bladder function improved after pretreatment with solifenacin alone: compliance; the number of control and obstructed rabbits showing OAB; the amplitude and frequency of OAB in control and obstructed rabbits; and contractile responses to 32 Hz FS, carbachol, and KCl. The following parameters of bladder function improved after pretreatment with α-LA: compliance; the number of obstructed rabbits showing OAB; the amplitude and frequency of OAB in control and obstructed rabbits; and contractile responses to 8 Hz FS and 32 Hz FS, carbachol, and KCl. The following parameters of bladder function improved after pretreatment with a combination of solifenacin, CoQ10, and α-LA: compliance; the contractile response to in situ Ach; the number of obstructed rabbits showing OAB; the amplitude of OAB of control

Figure 2. In situ response of the rabbit bladder to intra-arterial acetylcholine. Each bar is the mean ± standard error of mean of six individual rabbits. *Significantly different from the control; **significantly different from no-treatment group.

Figure 3. Contractile responses to field stimulation, carbachol, and potassium chloride. Each bar is the mean ± standard error of mean of six individual rabbits.

Figure 4. Effect of treatment on the contractile responses to field stimulation, carbachol, and potassium chloride. Data for the obstructed groups are presented as a percent of the control. Each bar is the mean ± standard error of mean of six individual rabbits. *Significantly different from the control; **significantly different from the no-treatment group.
and obstructed rabbits; the frequency of OAB in obstructed rabbits only; and contractile responses to 2 Hz FS, 8 Hz FS, and 32 Hz FS; carbamol; and KCl.

5. Conclusion

Our findings indicated that all three forms of treatment were effective in improving obstructed bladder function and reducing the level of OAB in both control and obstructed rabbits. The solifenacin-only and antioxidant-only groups were similar in their effectiveness, but the combination of solifenacin and antioxidants was more effective in improving more parameters than either of the 2 therapies alone, thus supporting our original hypothesis. Although our studies used an outcome obstruction model of OAB, we believe that the combination of solifenacin and antioxidants would be effective against nonobstructive OAB. This idea is supported by studies that have shown that nonobstructive OAB can be treated by antiuromacrine agents and antioxidants.

Acknowledgments

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