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Effect of coenzyme Q10 and α -lipoic acid on the response of the rabbit urinary bladder to repetitive stimulation and in vitro ischemia

Wei-Yu Lin^{1,2}, Alexandra Rehfuss², Catherine Schuler⁴, and Robert M. Levin^{2,3,4}

¹ Division of Urology, Chang Gung Memorial Hospital, Chia-Yi, Taiwan

² Albany College of Pharmacy, Albany, NY

³ Albany Medical College, Albany, NY

⁴ Stratton VA Medical Center, Albany, NY

Abstract

Objectives—The objective of these studies was to determine the efficacy of coenzyme Q10 (CoQ 10) and α -lipoic acid (α -LA), either alone or in combination, to protect the contractile responses of the rabbit urinary bladder from damage caused by repetitive stimulation in the presence or absence of in vitro ischemia.

Methods—Four groups of New Zealand White rabbits (N=4 per group) were treated with vehicle (group 1), CoQ 10 (group 2), α -LA (group 3), or CoQ 10 + α -LA (group 4) for 2 weeks. At the end of the treatment period, eight longitudinal strips from each rabbit bladder body were placed in oxygenated Tyrode's solution with glucose (normal physiological medium). Strips were stimulated by field stimulation (FS), carbachol, and KCl, and the responses were recorded. One half the strips were switched for 1 hour to Tyrode's with no glucose equilibrated with nitrogen (ischemia medium). Simultaneously, all strips were subjected to 1 h of repetitive FS (RS) followed by 1 h of recovery in normal physiological medium and the responses to all stimuli were recorded again.

Results—CoQ 10 showed no protective effect. α -LA resulted in increased contractile responses of the control bladder and showed a moderate protective effect for all forms of stimulation. The combination however, showed a significantly greater increased in the contraction of control bladder and a greater protective effect than α -LA alone

Conclusion—The combination of α -LA and CoQ10 treatment both enhanced contractile response in normal medium, and diminished contractile dysfunction induced by RS and ischemia.

Introduction

Partial bladder outlet obstruction (PBOO) is one of the major pathological effects of benign prostatic hyperplasia in men. In a variety of animal models of PBOO, it is well demonstrated that ischemia followed by reperfusion is one of the primary etiologies of progressive bladder dysfunction.^{1,2,3} In published studies, ischemia-reperfusion (I/R) induced the generation of

Corresponding Author: Robert M. Levin Ph.D., Albany College of Pharmacy, 106 New Scotland Ave, Albany, NY 12208, 518-445-7306, 518-445-7248, (fax) levinr@acp.edu.

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free radicals, which resulted in damage to the cellular and subcellular membranes; especially those of nerves and mitochondria.⁴ In addition, previous studies have demonstrated that natural products (Kohki tea, grapes, Edaravone, and pygeum africanum) with strong antioxidant and membrane protective properties has protective effects against the damage mediated by both PBOO and in vivo and in vitro models of I/R^{5,6,7}. In the current study, we evaluated the efficacy of the administration of two popular antioxidant agents, coenzyme Q10, alpha lipoic acid, on the response of the bladder to both repetitive stimulation and in vitro ischemia.

Coenzyme Q10 (CoQ10) is a lipid-soluble antioxidant and a key component of the mitochondrial electron transport chain for adenosine triphosphate (ATP) production.^{8,9} Previous experimental work has shown that CoQ10 has the ability to protect the myocardium against I/R injury and against aerobic pacing stress, especially in elderly hearts.^{10, 11} Preoperative oral coenzyme CoQ10 therapy in patients undergoing cardiac surgery increases myocardial and cardiac mitochondrial coenzyme CoQ10 levels, improves mitochondrial efficiency, and increases myocardial tolerance to in vitro hypoxia-reoxygenation stress.¹²

Alpha lipoic acid (α -LA) is found naturally within the mitochondria and is an essential cofactor for pyruvate dehydrogenase and α -ketoglutarate dehydrogenase.¹³ α -LA, reduced -LA (dihydrolipoic acid), and their metabolites also function as antioxidants. α -LA has also been reported to be an effective glutathione substitute, capable of increasing cellular glutathione levels and further improving the antioxidant status of the myocardium.¹⁴ α -LA has been demonstrated to protect a variety of membrane systems from oxidative injury including neuronal membranes, red blood cell membranes, and mitochondrial membranes.¹⁵ α -LA protects against hepatic I/R injury in rats.¹⁶ α -LA administered before torsion of the spermatic cord showed significant protective effects against I/R injury by decreasing lipid peroxidation and regulating testicular reduced glutathione and plasma total antioxidant power levels¹⁷. Also, previous study demonstrated that α -LA could ameliorate bladder contractile dysfunction enhanced by ethanol¹⁵.

In vitro studies demonstrated that the rate of development of contractile dysfunction in isolated bladder strips is significantly greater in the presence of an in vitro model of ischemia (hypoxia + glucose deprivation) than in the presence of hypoxia or glucose deprivation.¹⁸ Also, in in vitro models of ischemia, repetitive stimulation alone can increase contractile failure and increase lipid peroxidation; and also enhance the contractile damage induced by in vitro ischemia (incubation in the absence of oxygen and glucose).⁶ The time courses of the effect of repetitive stimulation in the presence and absence of both glucose deprivation and in vitro ischemia have been published previously.^{19, 20} The aim of the current study was to investigate whether CoQ10, α -LA, or the combination can protect against the contractile dysfunctions induced by repetitive stimulation in the presence or absence of in vitro ischemia.

Materials and Methods

Experimental Protocol

All methodologies were approved by the Institutional Animal Care and Use Committee of the Stratton VAMC. Sixteen adult New Zealand White male rabbits (3–5 kg) were obtained from Millbrook Breeding Labs (Amherst, MA). Animals were divided into four groups (N=4 per group) which were labeled as no treatment, CoQ10, α -LA and CoQ10 + α -LA. The no treatment group was fed with normal diet and treated with vehicle. The treatment groups were fed with normal diet but were supplemented orally with either CoQ-10 (3.0 mg/kg/day), α -LA (100 μ M/kg/day) or a combination for 2 weeks.

Tissue Preparation and Equilibration in Normal Oxygenated Tyrode's Solution

At the end of the two week period, each rabbit was sedated with ketamine/xylazine (25 mg ketamine, 10 mg xylazine/kg, i.m.); surgical anesthesia was maintained with intravenous pentobarbital (25 mg/kg). The urinary bladder was rapidly removed through a lower midline incision; the bladder body was separated from the base at the level of the ureteral orifices. Eight longitudinal muscle strips (full thickness) were cut from the bladder body. Each strip (70 ± 0.1 mg) was mounted in a separate 15 ml bath containing Tyrode's solution (NaCl 124.9 mM, KCl 12.5 mM, NaHCO₃ 23.8 mM, MgCl₂·6H₂O 0.5 mM, NaH₂PO₄·H₂O 0.4 mM, and CaCl₂ 1.8 mM) with glucose (1 mg/ml), equilibrated with 95% O₂, 5% CO₂ (normal physiologic medium), and maintained at 37°C. An initial tension of 1.0 g was placed on each strip and was equilibrated for at least 30 minutes. At the end of the preincubation period, each strip was stimulated by electrical FS with 20-second trains of 80-V, 1-ms pulses at three frequencies (2, 8, and 32 Hz) with 3-minutes interval between stimulus trains. Each strip was then stimulated with carbachol (20 μM), washed three times with fresh oxygenated buffer at 10-minute intervals, and stimulated with KCl (120 mM) followed by an additional three washes. The strips were exposed to KCl or carbachol for 3 minutes. Contractile responses were recorded using a Grass polygraph and digitized using a Polyview digital conversion system.

Methods for Producing an in Vitro Model of Ischemia and Repetitive Stimulation

Next, one half the strips were subjected to in vitro ischemia for 1 hour. An in vitro model of ischemia was produced by changing the aeration to 95% nitrogen and 5% carbon dioxide and changing the buffer to Tyrode's without glucose ("ischemia" medium). Simultaneously, all strips in both normal physiological medium or ischemia medium were stimulated repetitively for 20 seconds with 32-Hz FS at 5-minute intervals for 1 hour. At the end of the hour, all strips were washed and placed in normal physiologic medium for an additional hour without repetitive stimulation. At the end of this recovery time, a second set of contractile responses were recorded. (1 mg/ml), equilibrated with 95% O₂, 5% CO₂ (normal physiologic medium), and maintained at 37°C. An initial tension of 1.0 g was placed on each strip and was equilibrated for at least 30 minutes. At the end of the preincubation period, each strip was stimulated by electrical FS with 20-second trains of 80-V, 1-ms pulses at three frequencies (2, 8, and 32 Hz). Each strip was then stimulated with carbachol (20 μM), washed three times with fresh oxygenated buffer at 10-minute intervals, and stimulated with KCl (120 mM) followed by an additional three washes. Contractile responses were recorded using a Grass polygraph and digitized using a Polyview digital conversion system.

Statistical Analysis

The contractile responses were normalized to 100-mg tissue mass. Data are expressed as means $\pm$ SEM. Statistical analysis was done using 1- or 2-way ANOVA followed by Bonferroni's multiple range test for. $P < 0.05$ was required for significance.

Results

Figure 1 displays effect of treatments on the response to FS. Premedication with CoQ-10 did not have any effect on the contractile responses to FS under control conditions (fig 1A), following RS (fig 1B), or following RS + Ischemia (Fig 1C). Premedication with α -LA resulted in mild but significant increased contractile responses under control conditions and following RS. Premedication with the combination of α -LA + CoQ10 resulted in significant increases in the responses to FS under all three conditions. Furthermore, the combination therapy resulted in significantly greater responses than that following α -LA alone.

Figure 2 displays effect of treatments on the responses to carbachol and KCl. Premedication with CoQ-10 did not have any effect on the contractile responses to either form of stimulation

under control conditions (fig 2A), following RS (fig 2B), or following RS + Ischemia (Fig 2C). Premedication with α -LA resulted in mild but significant increased contractile responses under control conditions and following RS + ischemia. Premedication with the combination of α -LA + CoQ10 resulted in significant increases in the responses to both forms of stimulation under all three conditions. Furthermore, the combination therapy resulted in significantly greater responses than that following α -LA alone.

Discussion

Our results demonstrated that CoQ10 treatment was not significantly beneficial to contractile response in any situation, while α -LA treatment enhanced contractile response to various forms of stimulation and ameliorated the reduction in contractile response induced by repetitive stimulation. In addition, the current study confirmed that the combination therapy yielded better results than either monotherapy under all situations.

Previous studies demonstrated that supplementation of CoQ10 could bypass defects in the electron transport chain to maximize ATP production.²¹ As we know, mitochondrial enzyme function has an important role in the energy production and contractility of detrusor muscle.²² Impairment of mitochondria in both structure and function is a feature in contractile dysfunction secondary to PBOO.²² Mitochondria dysfunction mediated by I/R injury resulted in a number of cellular consequences including: (1) decreased ATP production; (2) increased reliance on alternative anaerobic energy sources; and (3) increased production of reactive oxygen species (ROS).²³ However, our study on CoQ10 supplementation did not show the anticipated effects. Further, different from previous studies about cardioprotection effects of CoQ10 treatment for myocardium in in vitro I/R^{12, 24}, CoQ10 treatment did not show any protection effect in bladder strip in the current study. Yet, our findings are entirely consistent in not only field stimulation (which reflects nerve function) but also carbachol and KCl (which reflect muscarinic receptor stimulation and smooth muscle contraction) responses. The possible reasons could be the difference in species (human, rat vs. rabbit), supplemental dosage (10mg/kg/day vs. 3.0mg/kg/day), and tissues (myocardium vs. bladder muscle).

Interestingly, α -LA treatment causes a positive inotropic effect on bladder strips in normal physiological medium. Previous study demonstrated that α -LA treated aged rats showed an increase in the status of dopamine, serotonin and norepinephrine. The results of previous study provide evidence that α -LA treatment can improve neurotransmitters during ageing.²⁵ Hence, we assessed that α -LA could act as a potent neuromodulator and contributed to the release of catecholamines from adrenergic stores, with a consequently marked increase in the force of contraction.

Also, we assessed that protective effect of α -LA on contractile dysfunction could be associated with modulation of calcium concentration. Intracellular Ca²⁺ overload with reduced activity of sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) is a critical mechanism for contractile dysfunction associated with I/R injury in myocardium and bladder.^{4, 26} Some study showed α -LA can increase Ca²⁺-ATPase activities in high glucose-treated human erythrocytes.²⁷ Therefore, α -LA could also any protection effect in bladder strip in the current study. Yet, our findings are entirely consistent in not only field stimulation (which reflects nerve function) but also carbachol and KCl (which reflect muscarinic receptor stimulation and smooth muscle contraction) responses. The possible reasons could be the difference in species (human, rat vs. rabbit), supplemental dosage (10mg/kg/day vs. 3.0mg/kg/day), and tissues (myocardium vs. bladder muscle). Interestingly, α -LA treatment causes a positive inotropic effect on bladder strips in normal physiological medium. Previous study demonstrated that α -LA treated aged rats showed an increase in the status of dopamine, serotonin and norepinephrine. The results of previous study provide evidence that α -LA treatment can improve neurotransmitters during

ageing.²⁵ Hence, we assessed that α -LA could act as a potent neuromodulator and contributed to the release of catecholamines from adrenergic stores, with a consequently marked increase in the force of contraction. Also, we assessed that protective effect of α -LA on contractile dysfunction could be associated with modulation of calcium concentration. Intracellular Ca^{2+} overload with reduced activity of sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) is a critical mechanism for contractile dysfunction associated with I/R injury in myocardium and bladder.^{4,26} Some study showed α -LA can increase Ca^{2+} -ATPase activities in high glucose-treated human erythrocytes.²⁷ Therefore, α -LA could also block the increase in free calcium mediated by *in vitro* ischemia.

One of our striking findings is that though CoQ10 group did not show any significantly beneficial effect, combination with α -LA group demonstrated to be advanced than α -LA group. An explanation for synergetic effect could be that the combination therapy could diminish one or more of the final common pathways of mitochondrial dysfunction and further improved mitochondrial ATP production. Our assessment was supported by previous study that the combination therapy (creatine monohydrate, coenzyme Q10, and lipoic acid) resulted in lower resting plasma lactate and urinary 8-isoprostanes, indicating that combination therapies targeting multiple final common pathways of mitochondrial dysfunction favorably influence surrogate markers of cellular energy dysfunction.²³

In addition to improve ATP synthesis, α -LA in combination with CoQ10 treatment therapy could further reverse some of the damage caused by decreased cellular energy availability and perhaps reducing oxidative stress caused by the impairment of the respiratory chain.²⁷ Our previous studies using the model of *in vitro* ischemia demonstrated that the contractile dysfunctions induced are directly related to the generation of free radicals (ex, Reactive oxygen species) and increased lipid peroxidation and that repetitive stimulation during the period of ischemia enhanced both the contractile dysfunction and lipid peroxidation.¹⁵ Reactive oxygen species can be scavenged in an aqueous environment by small molecules such as vitamin C, reduced glutathione (GSH), and uric acid. Previous study demonstrated that the pretreatment with α -LA reduced lipid peroxidation and increased GSH levels¹⁷; CoQ 10 is known as an antioxidant that directly scavenges radicals.^{23,29,30} Thus, combinations of these two antioxidant agents were more effective than individual antioxidant agent in protecting against oxidative stress. This was not surprising, because different antioxidants can replenish one another, thereby increasing the pool of total antioxidant power available to respond to the sudden surge of free radicals and reactive oxygen species after radiation exposure.^{31,32}

Conclusion

The current study demonstrated that combination of CoQ 10 and α -LA cause a positive inotropic effect in normal physiological medium and diminished the contractile dysfunction induced by repetitive stimulation with or without presence of *in vitro* ischemia. Further *in vivo* study would be indicated to investigate whether combination of CoQ 10 and α -LA could be a promising agent against PBOO -induced adverse effects on contractile function.

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Fig. 1.

a: Contractile responses to FS (2,8,32Hz) in normal physiologic medium. **b:** Effect of exposure to 1 hour repetitive stimulation in normal physiologic medium and **c:** after repetitive stimulation during 1 hour of in-vitro ischemia followed by 1 hour recovery. Each bar indicates mean $\pm$ SEM four individual rabbits.

*Significantly different from no treatment group (control), $P < 0.05$

Fig. 2.

a: Contractile responses to carbachol and KCl in normal physiologic medium. **b:** Effect of exposure to 1 hour repetitive stimulation in normal physiologic medium and **c:** after repetitive stimulation during 1 hour of in-vitro ischemia followed by 1 hour recovery. Each bar indicates mean $\pm$ SEM four individual rabbits.

*Significantly different from no treatment group (control), $P < 0.05$