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Original Article

Effect of coenzyme Q10 on ATP metabolism in red blood cells and cardiovascular hemodynamics in an awaken rat model

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ABSTRACT

Coenzyme Q10 (CoQ10) is a naturally occurring anti-oxidant increasingly used as a dietary supplement to enhance cardiovascular health. The objective of the current research was to study the effect of CoQ10 on cardiovascular hemodynamics and ATP metabolism in the red blood cells (RBC). Normotensive Sprague Dawley rats (SDR) with an implanted carotid artery catheter, weighing between 250 and 300 g were each housed in a freely moving caging environment with free access to drinking water. CoQ10 was dissolved in a vehicle made up of dimethyl sulfoxide (DMSO) and normal saline (1:1) for injection (5 mg/mL). Each rat received 4 doses of either 10 mg/kg of CoQ10 or the vehicle (n = 8 in each group) twice daily by subcutaneous (sc) injection. Blood samples (0.3 mL each) were collected before the injection of the last dose (T0), and at 0.08, 0.25 and 1 hour after the last dose for measurement of RBC concentrations of ATP and its catabolites by a validated high performance liquid chromatography assay (HPLC). Hemodynamic recordings were collected continuously throughout the experiment. Data between the two groups were compared and differences considered significant at $p < 0.05$ (Student's t-test). Systolic and diastolic blood pressure (SBP and DBP) and heart rate (HR) before the last CoQ10 injection was significantly lower in the CoQ10 treatment group (SBP 124 ± 3 vs 137 ± 3 mm Hg; DBP 101 ± 3 vs 111 ± 4 and HR 414 ± 6 vs 440 ± 7 bpm ($p < 0.05$)). The RBC concentrations of ATP were higher in the CoQ10 treatment group (1.97 ± 0.28 vs 1.13 ± 0.23 mM, $p < 0.05$). However, the acute effect on hemodynamics or RBC ATP concentrations measured up to 1 hour after the last injection was not significant ($p > 0.05$ by paired t-test). It was concluded CoQ10 decreased BP and HR and increased RBC concentrations of ATP after multiple doses in normotensive rats.

KEYWORDS: coenzyme Q10, ATP, hemodynamics, anti-oxidants, cardiovascular, rats.

INTRODUCTION

Cardiovascular disease including stroke is the leading cause of death and disability worldwide and an enormous economic burden to our societies [1]. It accounts for more than 17.3 million deaths per year globally which is expected to grow to more than 23.6 million by 2030 [2]. Based on the recent statistics released for heart and stroke disease, an estimated 83,600,000 adults in the United States (US) (>30%) have one form or another of cardiovascular disease (CVD), including more than 90% with hypertension, 18% with coronary heart disease (CHD), close to 10% with myocardial infarction (MI) and 8% with stroke. The estimated total direct and indirect cost in the

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US alone for treatment of CVD (hospitalization, drugs, home healthcare, etc.) and loss of productivity and morbidity is greater than 315 billion USD per year [2, 3]. Thus prevention and early control of the major CV risk factors could provide huge savings in health care costs worldwide. Despite major advances in treatment of CVD, the prevalence of hypertension, ischemic heart disease (IHD) and stroke is still on the rise. Identifying optimal strategies for cardiovascular prevention and to slow disease progression remains a therapeutic challenge.

In addition to diet and exercise, natural products and anti-oxidants are increasingly used in societies to enhance health and for prevention of chronic diseases [4-13]. Coenzyme Q10 (CoQ10) is a naturally occurring anti-oxidant which may have significant cardiovascular protective effect [14-19]. It is a ubiquinone involved in mitochondrial energy production and preserving membrane integrity resulting in increased ATP synthesis [15, 17, 19, 20]. It has been shown to have beneficial effects in patients with coronary artery disease (CAD), congestive heart failure (CHF) [15, 17, 21, 22], hypertension [23-26], neuromuscular and neurodegenerative disorders [27] [28] and migraine [29]. Patients who have lower serum CoQ10 concentrations have poorer prognosis from CHF [27], and those on long term statin therapy may have decreased plasma CoQ10 concentrations as a result of inhibition of HMG-CoA reductase. These patients may require CoQ10 supplementation to reduce the risk of statin-induced myopathy [14, 30-33]. On the other hand, it has been suggested that CoQ10 may interfere with the cardioprotective effect of the statins [34], implying that use of CoQ10 is not warranted. There are also evidence to suggest CoQ10 may be used to enhance the therapeutic effects of conventional cardiovascular medicines such as the statins, anti-hypertensive agents and perhaps others as well [23, 35], and to reduce cardiotoxicity induced by anti-cancer drugs without interfering with their anti-tumor effect [36, 37]. The controversy and conflicting opinion clearly requires further investigation. While the mechanism for the protective effect is not fully understood, it could be related to the anti-oxidant, anti-inflammatory and anti-ischemia properties of CoQ10 [21, 38], which are important contributing factors for ischemia preconditioning and cardiovascular protection. Deficiency in CoQ10 could lead to mitochondrial and vascular endothelial dysfunctions resulting in CV and metabolic diseases [18, 25, 39, 40]. Furthermore, there are evidence CoQ10 may inhibit first pass metabolism by cytochrome P-450 isozymes (CYP450), suggesting that it may be used as a pharmaceutical adjunct to enhance oral drug absorption and improve safety and efficacy profiles of many cardiovascular drugs [41, 42].

We have shown in several experimental animal models ATP metabolism in the RBC is a potential biomarker for cardiovascular protection and possibly also a surrogate measure of the inner energy in the body [43-45]. In light of the potential benefit of CoQ10 on energy metabolism and lowering blood pressure [19, 26, 46, 47], the current study was carried out to investigate the effect of CoQ10 on cardiovascular hemodynamics and ATP metabolism in the RBC using an awaken rat model as described previously [44].

MATERIALS AND METHODS

Chemicals

CoQ10 was received as gift from Ocean Nutrition Canada (Halifax, NS, Canada). Authentic standards of purine nucleotides, nucleosides and oxypurine metabolites including ATP, adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), and the internal standard 3,7-dimethyluric acid (DMUA) used in the HPLC assay were purchased from Sigma-Aldrich Chem Co. (St. Louis, MO, USA). Solvents were HPLC grade, and all other chemicals were reagent grade (Fisher Scientific, ON, Canada).

Animal study

The protocol followed the Canadian Council on Animal Care guidelines and was approved by the Dalhousie University Committee on Laboratory Animals. Male Sprague Dawley (SD) rats weighing between 250-300 g with a silastic catheter implanted into a carotid artery were purchased from Charles River Laboratories (Wilmington, MA, USA). They were acclimatized for one week with free access to food and water *ad libitum* before experiment. CoQ10 was dissolved in a

vehicle made up of dimethyl sulfoxide (DMSO) and normal saline (1:1) for injection (5 mg/mL). Each rat was randomized to receive 4 doses of either 10 mg/kg of CoQ10 (T Group, n = 8) or the vehicle (C group, n = 8) twice daily by subcutaneous (sc) injection. The rats were kept in a cage with free access to drinking water during experiment (Figure 1). Blood samples (0.3 mL each) were obtained from each rat from the carotid artery catheter using a swivel-tether system (Figure 1) before the injection of the last CoQ10 dose and then at 0.08, 0.25 and 1 hour after the injection of the last CoQ10 dose for measurement of RBC concentrations of ATP by a previously published HPLC method [48]. The blood samples collected were immediately mixed with a "Stopping Solution" and centrifuged at 1760 x g at 4 °C for 10 min to separate plasma from the RBC. The RBC was lysed with ice cold 10% tricholoroacetic acid to obtain lysates for measurement of ATP, ADP and AMP by HPLC as described previously [48].

Figure 1. Awaken rat model.

Hemodynamic recordings including systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were collected from the carotid artery catheter continuously using a TruWave disposable pressure transducer (Model PX601, Edwards Lifesciences Canada, Inc., Mississauga, ON, Canada) coupled to a Siemens hemodynamic monitor (Sirecust 400) and chart recorder (Siredoc) (Erlangen, FRG) as previously described [49-53]. The rats were euthanized by cardiac puncture at the end of the experiment under general anaesthesia with isoflurane.

Data analysis

Areas under the curve (AUC) of RBC concentrations of ATP and other adenine nucleotides (i.e. ADP and AMP) were calculated using the trapezoidal method (Prism®-5, Graphpad Software Inc., La Jolla, USA). RBC concentrations of the adenine nucleotides and hemodynamic parameters between the control and CoQ10 treatment groups during the experiment were analyzed by *student's unpaired t-test*. A *paired t-test* was used to compare the biomarker data before and after the last injection in the same rat and differences were considered significant when $p < 0.05$ (Minitab® Inc., Release 18, State College, PA, USA).

RESULTS

Following 3 subcutaneous (sc) injections over 2 days, SBP and DBP before the last CoQ10 injection (T0) was 124 ± 3 and 101 ± 3 mm Hg which were significantly lower than those in the vehicle control group (137 ± 3 and 111 ± 4 mm Hg, respectively ($p < 0.05$ for both)). HR was also lower in the CoQ10-treated group (414 ± 6 vs 440 ± 7 bpm, $p < 0.05$) (Figure 2). The RBC concentrations of ATP before the last CoQ10 injection (T0) were significantly higher in the CoQ10-treated group (1.97 ± 0.28 vs 1.13 ± 0.23 mM, $p < 0.05$). On the other hand, the ADP concentrations in the RBC were significantly lower in the CoQ10-treated group (0.41 ± 0.06 vs 0.64 ± 0.07 mM, $p < 0.05$) (Figure 3). The ratios of ATP/ADP and ATP/AMP measured at T0 were significantly higher than those in the control group (Figure 4) suggesting CoQ10 increased metabolism of ATP in the RBC after repeated administration.

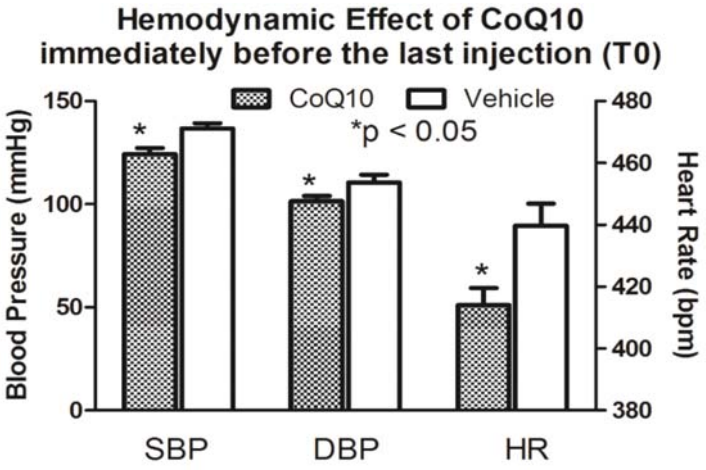

Figure 2. Hemodynamic effect of CoQ10 at steady state.

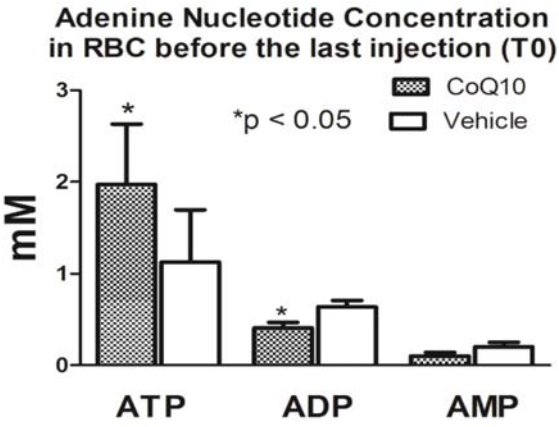

Figure 3. RBC adenine nucleotide concentrations at steady state.

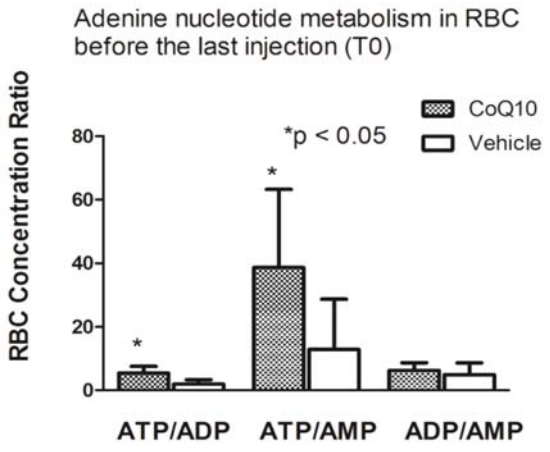

Figure 4. RBC adenine nucleotide metabolism at steady state.

On the other hand the acute effect of CoQ10 immediately following injection was less apparent. Except for the RBC concentration of ADP which was significantly lower after the last injection of CoQ10, there was no significant difference between the hemodynamic effect or RBC concentrations of the other adenine nucleotides (ATP or AMP) measured 1 hour after the last injection ($p > 0.05$ by paired t-test) (Figures 5 and 6). Increase in ATP metabolism in the RBC was also less apparent when it was measured 1 hour after the last CoQ10 injection. We noted only a significant increase of the AUC ratio of ATP to ADP (but not ATP/AMP) in the RBC from T0 to T1 (i.e. one hr after the last dose) in the CoQ10-treated group (6.40 ± 1.02 vs 3.50 ± 0.81, $p < 0.05$) suggesting CoQ10 also increased metabolism of ATP in the RBC shortly after the last injection (Figure 7), albeit that the acute effect was considerably less than the effect at steady state observed before the last injection.

DISCUSSION

There is a general consensus that CoQ10 supplementation may have significant health benefits [14-19] particularly in patients with cardiovascular diseases. Although the mechanism for its health benefit is still not clear, it is believed to be mediated by enhancing mitochondrial energy metabolism and preserving membrane integrity which results in increased ATP synthesis [15, 17, 19, 20]. While there is as yet no direct evidence linking increased ATP metabolism in the

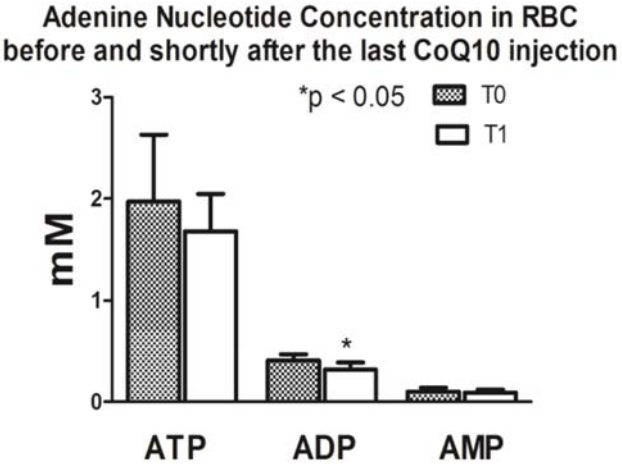

Figure 5. RBC adenine nucleotide concentrations one hour after the last injection of CoQ10.

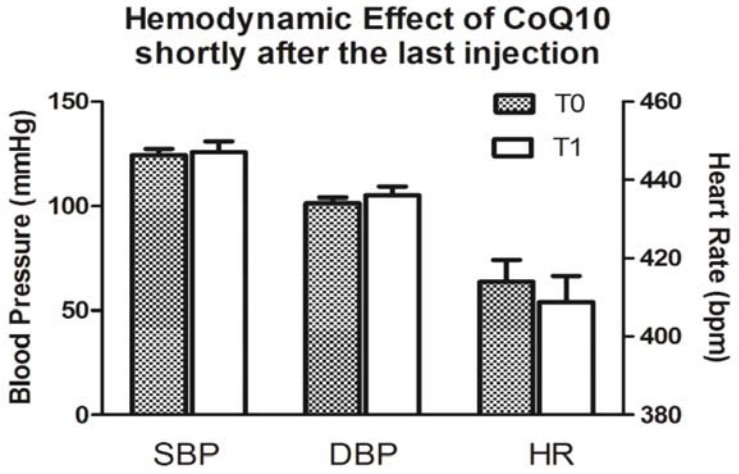

Figure 6. Hemodynamic effect of CoQ10 one hour after the last dose.

RBC to cardiovascular health, the result from the current experimental study in rats has demonstrated for the first time repeated administration of 4 doses of CoQ10 (10 mg/kg) twice daily increased ATP metabolism in RBC (Figure 4).

ATP metabolism in RBC has been shown to be a potential surrogate biomarker for post-exercise hypotension and cardiovascular protection in experimental animal models [45]. In response to ischemia and cardiovascular injury, ATP is broken down to release adenosine. The effect of adenosine is very short-lived because it is rapidly taken up by RBCs, myocardial and endothelial cells, and also rapidly catabolized to oxypurine metabolites such as hypoxanthine and uric acid. When the energy level is restored, intracellular adenosine is phosphorylated back to adenine nucleotides *via* a salvage pathway. These metabolic events are known to occur in the myocardium, endothelium as well as in RBCs. We have shown previously acute exercise and anti-hypertensive agents such as diltiazem both increased ATP concentrations in the RBCs, which may be an important mechanism for their cardiovascular protective effect [44, 54]. The results from the current study suggest CoQ10 may have similar potential for cardiovascular protection, and that ATP metabolism in the RBC may be a surrogate for energy metabolism in the mitochondria and perhaps also in the body.

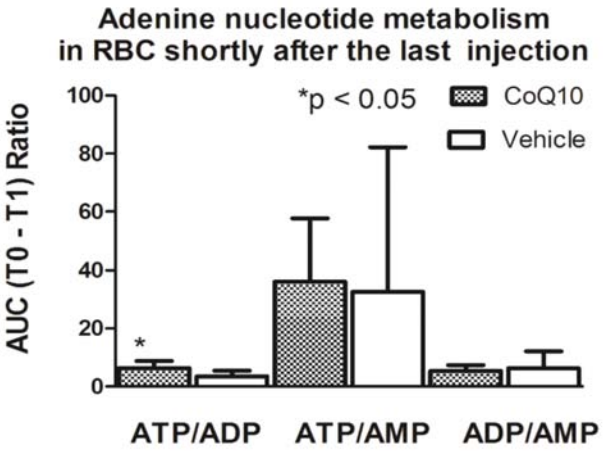

Figure 7. RBC adenine nucleotide metabolism one hour after the last injection of CoQ10.

Clinically, it has been suggested CoQ10 supplementation has potential to lower blood pressure which would be beneficial for patients with hypertension, angina and congestive heart failure, although the evidences are far from conclusive [25, 55, 56]. Studies using experimental rat models have shown CoQ10 had cardiovascular protective effect against acute myocardial infarction and heart failure induced by isoproterenol [57, 58] or by coronary artery occlusion [59]. These protective effects include improvement in left ventricular dysfunction, reduction in oxidative stress and ECG changes, enhanced autophagy, reduced myocardial apoptosis and cell death [59-61]. The current study is the first to demonstrate a significant attenuating effect on blood pressure (both SBP and DBP) and HR directly in normotensive SD rats (Figure 2). It is possible the hemodynamic effect is linked to the increased ATP metabolism in the RBC, which has been hypothesized as a possible mechanism for post-exercise hypotension and cardiovascular protection [45]. Future studies of the effect of CoQ10 on ATP metabolism in the RBC in a model of cardiovascular injury may further support the potential cardiovascular protective effect of CoQ10.

It is not clear why there was a significant difference in the effects observed before the last injection (T0) between the control and CoQ10 treatment groups, but yet no difference between the effects at T0 and those observed one hour after the last injection (T1). However we could speculate one of the reasons for the delay may be related to the relatively slow absorption and limited distribution of CoQ10 to the site of action following injection. It is well known CoQ10 is an insoluble, poorly permeable antioxidant [62]. Various drug delivery technologies such as using emulsification and nanoparticles are currently under development to improve the oral bioavailability of CoQ10 [62-65]. As a result of poor permeability, the effect of CoQ10 might be delayed such that it was greater when measured 12 hrs after the injection than the acute effect measured 1 hour after the injection.

CONCLUSION

In summary, the current study has demonstrated CoQ10 increased metabolism of ATP in the RBC and lowered blood pressure and HR in normotensive SD rats. Further study is warranted to explore its potential as a cardiovascular protective agent.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest with the current study.

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