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Oral pyrroloquinoline quinone (PQQ) during pregnancy increases cardiomyocyte endowment in spontaneous IUGR guinea pigs

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Brief Report

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Abstract

Background: Intrauterine growth restriction (IUGR) exerts a negative impact on developing cardiomyocytes and emerging evidence suggests activation of oxidative stress pathways plays a key role in this altered development. Here, we provided pregnant guinea pig sows with PQQ, an aromatic tricyclic o-quinone that functions as a redox cofactor antioxidant, during the last half of gestation as a potential antioxidant intervention for IUGR-associated cardiomyopathy.

Methods: Pregnant guinea pig sows were randomly assigned to receive PQQ or placebo at mid gestation and fetuses were identified as spontaneous IUGR (spIUGR) or normal growth (NG) near term yielding four cohorts: NG ± PQQ and spIUGR ± PQQ. Cross sections of fetal left and right ventricles were prepared and cardiomyocyte number, collagen deposition, proliferation (Ki67) and apoptosis (TUNEL) were analyzed.

Results: Cardiomyocyte endowment was reduced in spIUGR fetal hearts when compared to NG; however, PQQ exerted a positive effect on cardiomyocyte number in spIUGR hearts. Cardiomyocytes undergoing proliferation and apoptosis were more common in spIUGR ventricles when compared with NG animals, which was significantly reduced with PQQ supplementation. Similarly, collagen deposition was increased in spIUGR ventricles and was partially rescued in PQQ-treated spIUGR animals.

Conclusion: The negative influence of spIUGR on cardiomyocyte number, apoptosis, and collagen deposition during parturition can be suppressed by antenatal administration of PQQ to pregnant sows. These data identify a novel therapeutic intervention for irreversible spIUGR-associated cardiomyopathy.

Introduction

Intrauterine growth restriction (IUGR) affects 3–7% of pregnancies and is linked to significant morbidity in the perinatal period that may continue into adolescents and adulthood.[1] While low birth weight is not synonymous with IUGR, the current paradigm holds that programming for many adult-onset diseases are derived in-part from perturbations in development that arise in response to an adverse intrauterine or extrauterine environment.[2] In particular, diseases of the cardiovascular system, including atherosclerosis, hypertension, and heart failure, are over-represented in adults with low birth weight.[3,4] The underlying mechanisms for this programming are not fully understood, but oxidative stress appears to be a key feature of the *in utero* environment that leads to fetal growth restriction.[5-7]

Myocardial maturation is highly predictable. Proliferative mononucleated cardiomyocytes (CM) are common in the perinatal period and give rise to terminally differentiated bi-nuclear CMs.[8] This transition is accompanied by postnatal CM pruning and interference in this tightly regulated maturation process is linked to reduced CM investment, increased CM size, and impaired heart function in later life.[9,10] Unfortunately, this process appears to be irreversible and few therapeutic options are available for slowing the untoward effects of slow intrauterine growth on cardiac architecture and performance. In response to this gap in care, we randomly assigned pregnant guinea pig sows to receive the antioxidant pyrroloquinoline quinone (PQQ) in their drinking water from mid gestation and harvested fetal hearts near term to determine whether PQQ positively influences cardiac investment in a model of physiologic spIUGR.

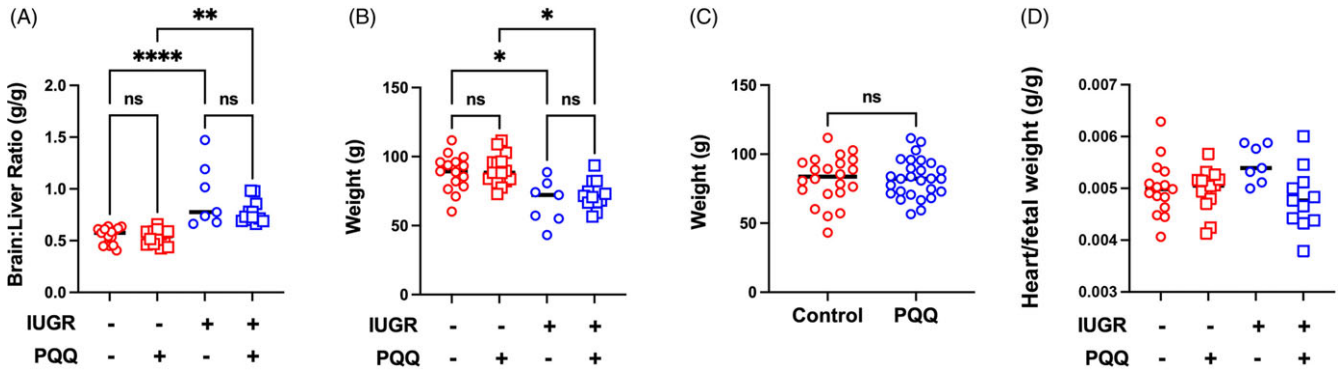

Fig. 1. PQQ does not alter fetal organ or total body weight. A-B. Total body weight (**A**) and brain:liver ration (**B**) for normal growth (red) and IUGR (blue) placebo (open circles) and PQQ-treated (open squares) fetuses. Data represent individual measurements, and bars represent mean value for each group. **C**. Total body weight for placebo (red circles) and PQQ treatment (blue circles) fetuses. NS = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Methods

Animals

All animal procedures were conducted in accordance with guidelines and standards of the Canadian Council on Animal Care. Animal Use Protocol (AUP-#2018-110) was approved, and post approval monitoring was conducted by the Western University Animal Care Committee. Time-mated pregnant Dunkin-Hartley guinea pigs (Charles River Laboratories, Wilmington, MA, USA) were purchased arriving at ~25 days gestation and housed in 12 h light and dark cycles in a temperature (20 ± 2° C) and humidity (30–40%) controlled environment, with access to guinea pig chow (LabDiet diet 5025) and tap water *ad libitum*. Sows were randomly assigned to placebo ($n = 4$) or PQQ ($n = 5$) in drinking water (1.5 mg/L, Sigma) beginning at day 35 of gestation and fetoplacental tissues were harvested at day 65 (Term ~69 days). The PQQ concentration corresponded with an average dose of 0.18 mg/day/kg, which was scaled from other studies in mice.[11,12]

Tissue collection and histology

Sows were euthanized via CO_2 inhalation. Upon confirmation of maternal death, the fetuses and their placentae were exposed and fetuses were euthanized. Fetal sex was determined and each fetus was weighed. Fetal organs were excised into ice-cold PBS, blotted dry, and weighed. Hearts were weighed, bisected into right ventricle and left ventricle, and a transverse section was collected, fixed, and embedded for histology as described previously.[9] Guinea pig fetuses with body weight <85 g and brain-liver ratio >0.65 were designated as IUGR and all other fetuses were designated as normal growth (NG).

H&E-stained sections of the left and right ventricular myocardium were analyzed in five separate 40X high-power fields (hpf) per sample using an Olympus inverted microscope equipped with a Nikon digital camera system. Cardiomyocytes (CM) were counted in five separate hpf per sample and mean value for each animal was calculated. Only cardiomyocytes where striations were clearly visible were counted.[9] Myocardial cross sections were stained for Ki67 and DNA strand break (TUNEL) and stained CMs were counted in five separate hpf per sample and quantified as previously described.[9] Collagen staining in each ventricle was determined using Mason's Trichrome and reported as percent area per high powered field.

Statistical analysis

Mixed linear models were created to analyze fetal data while considering non-independence due to shared litter effects. These models were used to investigate the associations between PQQ supplementation and intrauterine growth restriction as fixed effects on resulting fetal cardiomyocyte parameters, while controlling for litter-to-litter variation as a random effect. Fetal sex was initially considered as a fixed effect but was found negligible by an Analysis of Variance conducted on the initial models and thus was removed from the analysis. Analyses were executed in R version 4.1.3 using the lme4 package to create multilevel models with random effects. The lmerTest package was employed to conduct a Type III Analysis of Variance using Satterthwaite's method for estimating degrees of freedom. The emmeans package, which employs Tukey's multiple comparison test, was applied as a post hoc test to individual factors (ie. PQQ− vs. PQQ+) and subgroups of combined factors (ie. IUGR−/PQQ− vs IUGR−/PQQ+) of each mixed linear model when indicated by an Analysis of Variance p-value <0.05.

Litter and fetal characteristics

The average litter size was not significantly different between −PQQ and +PQQ sows averaging approximately five per group. We sought to designate a cohort of fetuses as spIUGR without artificially inducing poor intrauterine growth as used in other guinea pig models.[9,13-16] Using a weight cutoff (<85 g) and a brain-liver ratio (>0.65) to identify physiological IUGR fetuses, approximately 39% of fetuses were included in the spIUGR cohort and the remainder designated as Normal Growth (NG) to yield four cohorts; −PQQ (NG; 15 and spIUGR; 7) and +PQQ (NG; 15 and spIUGR; 12) groups (Fig. 1A and B). Fetal sex (Female/Male) was determined and while distribution was relatively well distributed in −PQQ/NG (8/7), +PQQ/NG (6/9) and +PQQ/+IUGR (5/7) groups, the −PQQ/+IUGR group was unbalanced (1/6). Importantly, +PQQ did not alter fetal weight (Fig. 1C) and heart weight indexed to body weight was similar between −PQQ and +PQQ NG and IUGR fetuses (Fig. 1D).

Similar to other models of IUGR, CM number was significantly reduced in both ventricles in spIUGR fetuses; however, PQQ treatment significantly increased CM number in NG ventricles with a more modest, but not statistically significant increase in CM number observed in spIUGR ventricles (Fig. 2A). Proliferating Ki67+ CMs were more frequently observed in both ventricles of spIUGR

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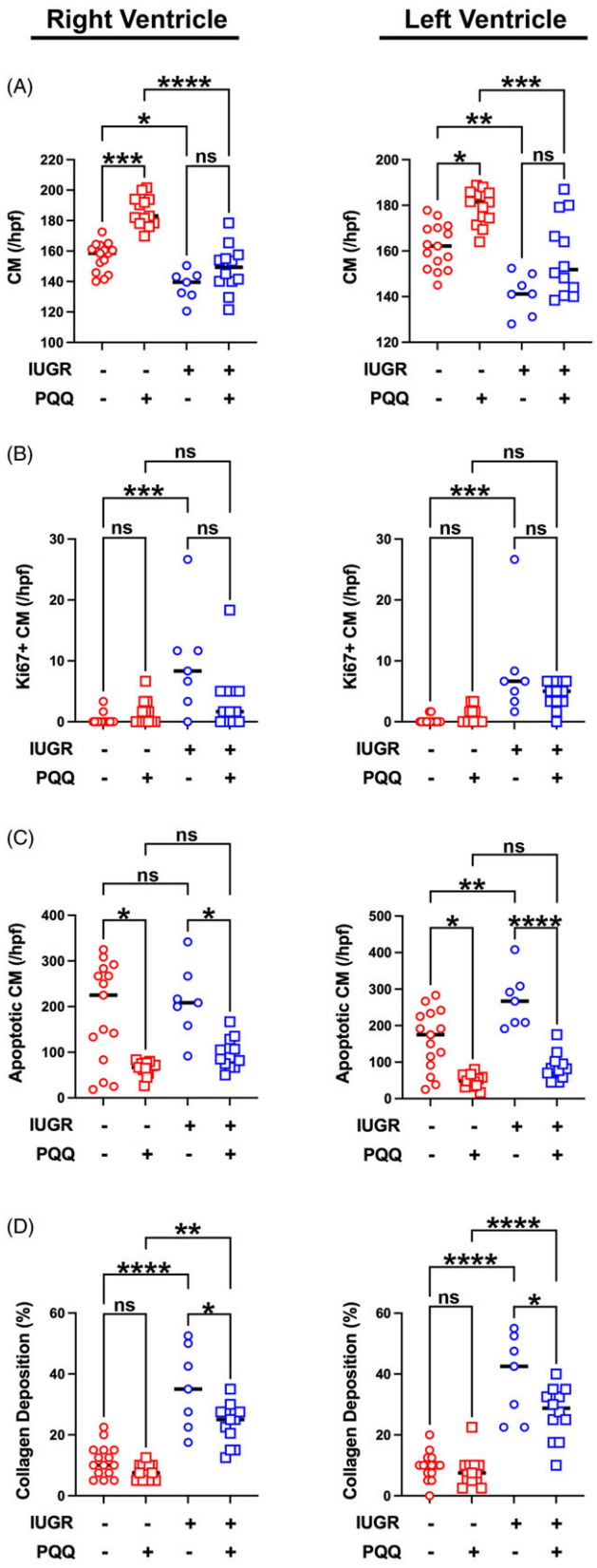

Fig. 2. PQQ increases cardiomyocyte number and reduces collagen deposition in IUGR hearts. A-D. Cardiomyocyte number (**A**), Ki67+ proliferative cardiomyocytes (**B**), apoptotic cardiomyocytes (**C**), and collagen deposition (**D**) in normal growth (red) and IUGR (blue) placebo (open circles) and PQQ (open squares) treated fetuses. Data represent number of cells (**A-C**) or percent staining (**D**) per high-power field (hpf), and bars represent mean value for each group. NS = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

hearts, but PQQ supplementation had no significant effect on Ki67+ CM number in either spIUGR and NG ventricles (Fig. 2B). The percent of mononucleated CM in the right and left ventricle did not differ between the NG and IUGR fetuses, nor the placebo or PQQ-treated groups (data not shown). Apoptotic CMs were commonly identified in both NG and spIUGR ventricles; however, the number of apoptotic CMs was significantly increased in the LV, but not the RV, of spIUGR hearts when compared with NG hearts (Fig. 2C). Strikingly, PQQ supplementation significantly reduced the number of apoptotic CMs in both ventricles from NG and spIUGR hearts (Fig. 2C). Both ventricles from spIUGR hearts displayed more prominent collagen deposition when compared with placebo-treatment ventricles and PQQ partially rescued this observation (Fig. 2D).

Discussion

Cardiac development proceeds in a predictable pattern that continues into postnatal life.[17] Slow intrauterine growth compromises CM maturation and performance with indications of abnormal cardiac morphology and function present in late fetal stages of development that persist into adulthood.[18] Regardless of the animal model employed, fetal growth restriction reduces CM investment by shifting the timing of CM pruning into the late fetal stage, which has lasting consequences for cardiac performance.[9,19]

PQQ is a redox cofactor that is purported to increase mitochondrial biogenesis, sequester free radicals, and stabilize the electron transport chain, all of which are attractive strategies to improve CM bioenergetics.[20-22] Similar to our results in an induced-IUGR guinea pig model,[9] spIUGR ventricles are characterized by decreased CM number and maternal supplementation with PQQ increases CM number in spIUGR ventricles. Further, PQQ appears to suppress CM apoptosis and proliferation as well as abnormal collagen deposition in IUGR hearts. Similar results were identified in normal growth ventricles suggesting that the actions of PQQ are not necessarily dependent on hypoxia or abnormal bioenergetics, which are characteristic of IUGR hearts. Importantly, PQQ does not affect fetal growth or heart mass. Given the dependence of cardiomyocytes on β-oxidation of fatty acids (FA) for ATP synthesis, which is dysregulated in multiple animal models of IUGR,[23-25] it is attractive to hypothesize that PQQ's effect on CM pruning and ventricular remodeling observed in our data is mediated through improved β-oxidation and energy production in IUGR hearts. It must be acknowledged that dysregulated FA metabolism may occur through multiple steps including down-regulation of transcellular and mitochondrial FA transporters as well as β-oxidation enzymes despite adequate supplies of plasma acylcarnitines in the fetal circulation.[24,25] Thus, the mechanism through which PQQ mitigates the untoward effects of fetal growth restriction on heart development and cardiomyocyte investment remain to be determined.

Perinatal PQQ administration appears efficacious in other models with impaired lipid metabolism. The offspring of mothers provided supplemental PQQ in a model of fatty liver disease had improved glucose handling and lipoprotein profile compared to control offspring.[12] These differences persisted into adulthood even after withdrawal of PQQ highlighting a disease mitigating window during the perinatal period. PQQ also increases proliferator-activated receptor alpha (PPARα) expression, a key transcriptional regulator of genes involved in lipid metabolism.[25] While our findings do not provide direct insight into mechanism, several

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other IUGR models feature dysregulated PPARα activity that corresponds with alterations in multiple FA metabolism pathways.

While our findings hold promise for reversing the undesirable effects of fetal growth restriction on CM architecture, suppression of CM apoptosis in NG ventricles warrants caution. CM apoptosis is highly regulated in the perinatal period and is necessary for heart maturation as evidenced by the high expression of apoptotic markers in both NG and spIUGR ventricles and in other models.[9,26,27] Suppression of CM apoptosis may impair the physiologic remodeling of the myocardium in response to shifting blood flow and exposure to higher ambient oxygen that accompanies birth. Ultimately, a targeted approach for maternal PQQ supplementation would be ideal but also requires identification of IUGR fetuses in order to avoid unwanted effects in normal growth infants. While necessary for translation of our findings, this approach may miss the most effective window for intervention.

In conclusion, we demonstrate that maternal PQQ administration fails to increase CM number significantly, but does suppress CM apoptosis and collagen deposition in spontaneous IUGR and normal growth hearts. These data provide evidence that therapies targeting mitochondrial biogenesis and FA metabolism may be effective strategies for IUGR-associated cardiomyopathy.

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Conflicts of Interest. The authors have no conflicts of interest to disclose.

Ethical standards. None.

Category. Translational.

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