Introduction

Barth syndrome (BTHS) and dilated cardiomyopathy with ataxia disease (DCMA) are inherited metabolic disorders biochemically characterized by tissue accumulation of 3-methylglutaconic (MGT) and 3-methylglutaric (MGA) acids\textsuperscript{1,2}. While BTHS is an X-linked recessive disorder caused by mutations in the TAZ gene, DCMA is a recessive disorder caused by mutations in the DNAJ Heat Shock Protein Family (Hsp40) Member C19 (DNAJC19) gene\textsuperscript{1,2}. Cardiomyopathy is the main clinical finding in both disorders and commonly lead to premature death\textsuperscript{1,2}. Since the pathophysiology of this abnormality is not fully established, we evaluated the in vivo effects of MGA on parameters of oxidative stress, bioenergetics, and mitochondrial biogenesis and dynamics in heart of rats, as well as important pathways associated with these processes. Vascular reactivity was further analyzed in the aorta of these animals. Additionally, we investigated the effects of bezafibrate (BEZ), a molecule that activates PPAR receptors and induces mitochondrial biogenesis, on MGA toxicity.

Methods

Thirty-day-old Wistar rats received three intraperitoneal injections of NaCl (control) or MGA (first injection of 10μmol/g followed by two of 5μmol/g). Treatment with BEZ (30 or 100mg/kg/day) was performed by gavage during seven days before MGA administration. The doses of MGA and BEZ were previously used by our group\textsuperscript{3}. The animals were euthanized 1h after last MGA injection and heart and aorta samples were prepared for the measurement of the parameters, as previously described\textsuperscript{3}.

A PPAR agonist prevents 3-methylglutaric-induced impairment of antioxidant defenses and mitochondrial dysfunction in rat heart

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Groups:

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<tr>
<th>Vehicle (corn oil)</th>
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<th>BEZ 30mg/kg/day + MGA</th>
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<td>Pre-treatment with BEZ</td>
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Results

Figure 1. Effect of 3-methylglutaric acid (MGA) and bezafibrate (BEZ30: 30 mg/kg/day; BEZ100: 100 mg/kg/day) administration on reduced glutathione (GSH) levels (A), superoxide dismutase (SOD) (B and C) and catalase (CAT) (D) activities and heme oxygenase-1 (HO-1) protein content (E) in rat heart. Values are expressed as means ± SD (n= 4 to 6). *P < 0.05, **P < 0.01, compared to controls; #P < 0.05, ##P < 0.01, compared to MGA groups (ANOVA followed by Duncan multiple range test).

Figure 2. Effect of 3-methylglutaric acid (MGA) and bezafibrate (BEZ100: 100 mg/kg/day) administration on the activities of the mitochondrial respiratory chain complexes II (CII) (A) and IV (CIV) (B) in rat heart. Values are expressed as means ± SD (n=4 to 6). *P < 0.05, compared to controls; #P < 0.05, compared to MGA group (ANOVA followed by Duncan multiple range test).

Figure 3. Effect of 3-methylglutaric acid (MGA) and bezafibrate (BEZ100: 100 mg/kg/day) administration on levels of dynamin-related protein-1 (Drp1) (A), mitofusin-1 (MFN1) (B), Akt (C), and Sirtuin-1 (Sirt1) (D) in rat heart. Values are expressed as means ± SD (n= 3 to 5). *P < 0.05, compared to controls; #P < 0.05, compared to MGA group (ANOVA followed by Duncan multiple range test).
Results

**Figure 8.** Effect of 3-methylglutaric acid (MGA) and bezafibrate (BEZ100: 100 mg/kg/day) administration on vascular response to phenylephrine (A) and acetylcholine (B) in aortic rings. Each point represents mean ± SD (n= 7 to 8). *P < 0.05: significant difference between control and MGA groups; &P < 0.05: significant difference between control and BEZ100+MGA groups (one-way ANOVA followed by Newman-Keuls multiple comparison test).

**Summary**

Our data showed that MGA administration induces oxidative stress in rat heart. MGA decreased superoxide dismutase and catalase activities, suggesting that free radicals generated by MGA oxidize critical amino acids of these enzyme structures. In line with this, we verified that MGA also reduced GSH levels, a crucial non-enzymatic antioxidant defense. We also observed that MGA disturbs bioenergetics by decreasing the activity of the respiratory chain complexes II and IV. Since oxidative stress and bioenergetic failure are commonly associated with alterations in mitochondrial quality control (da Rosa-Junior et al., Neurotox Res, 35:809-822, 2019), we also investigated the effects of MGA on mitochondrial biogenesis and dynamics. MGA increased DRP1 content, and decreased Sirt1 and Akt content, suggesting an increase in fission and a reduction in biogenesis. Moreover, MGA altered aorta vascular reactivity in the presence of phenylephrine and acetylcholine, that cause contraction and relaxation, respectively.

We found that BEZ prevented most alterations in the activity of antioxidant enzymes, and changes in CII activity, and DRP1, Sirt1 and Akt content, suggesting protective effects of this molecule. Although BEZ did not prevent vascular reactivity alterations, we cannot rule out that longer periods of treatment with BEZ may be effective against MGA toxicity. In conclusion, it may be presumed that oxidative stress and mitochondrial bioenergetics and control quality disturbances caused by MGA underlie the cardiac abnormalities observed in BTHS and DCMA.
References


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