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Research Report

2-Methylbutyrylglycine induces lipid oxidative damage and decreases the antioxidant defenses in rat brain

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ABSTRACT

Short/branched chain acyl-CoA dehydrogenase (SBCAD) deficiency is an autosomal recessive disorder of isoleucine metabolism biochemically characterized by accumulation of 2-methylbutyrylglycine (2MBG) and 2-methylbutyric acid (2MB). Affected patients present predominantly neurological symptoms, whose pathophysiology is not yet established. In the present study, we investigated the *in vitro* effects of 2MBG and 2MB on important parameters of oxidative stress in cerebral cortex of young rats and C6 glioma cells. 2MBG increased thiobarbituric acid-reactive species (TBA-RS), indicating an increase of lipid oxidation. 2MBG induced sulfhydryl oxidation in cortical supernatants and decreased glutathione (GSH) in these brain preparations, as well as in C6 cells, indicating a reduction of nonenzymatic brain antioxidant defenses. In contrast, 2MB did not alter any of these parameters and 2MBG and 2MB did not affect carbonyl formation (protein damage). In addition, 2MBG-induced increase of TBA-RS levels and decrease of GSH were prevented by free radical scavengers, implying that reactive species were involved in these effects. Furthermore, the decrease of GSH levels caused by 2MBG was not due to a direct oxidative action since this metabolite did not alter sulfhydryl content from a commercial solution of GSH. Nitric oxide production was not altered by 2MBG and 2MB, suggesting that reactive oxygen species possibly underlie 2MBG effects. Finally, we verified that 2MBG did not induce cell death in C6 cells. The present data show that 2MBG induces lipid oxidative damage and reduces the antioxidant defenses in rat brain. Therefore, it may be postulated that oxidative stress induced by 2MBG is involved, at least in part, in the pathophysiology of the brain damage found in SBCAD deficiency.

Abbreviations: DNPH, 2,4-dinitrophenylhidrazine; DTNB, 2-dithio-bis(2-nitrobenzoic acid); 2MBG, 2-methylbutyrylglycine; 2MB, 2-methylbutyric acid; ANOVA, Analysis of variance; GSH, glutathione; MEL, Melatonin; NEM, *N*-ethylmaleimide; SBCAD, Short/branched chain acyl-CoA dehydrogenase; TBA-RS, Thiobarbituric acid-reactive substances; TRO, Trolox; SPSS, Statistical Package for the Social Sciences

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1. Introduction

Short/branched chain acyl-CoA dehydrogenase (SBCAD; OMIM 600301/610006) deficiency, also known as 2-methylbutyryl-CoA dehydrogenase deficiency or 2-methylbutyrylglycinuria, is an autosomal recessive disorder recently reported that affects isoleucine catabolism. SBCAD is a homotetrameric mitochondrial enzyme that catalyzes the dehydrogenation of 2-methylbutyryl-CoA to tiglyl-CoA (Rozen et al., 1994; Andresen et al., 2000). The disorder is biochemically characterized by tissue accumulation and high urinary excretion of 2-methylbutyrylglycine (2MBG) and 2-methylbutyric acid (2MB). 2MBG generation occurs from the transesterification of 2MB with glycine by the enzyme acyl-CoA glycine *N*-acyltransferase, reaching levels of up to 100 μg/mg creatinine in the urine of affected patients (Matern et al., 2003).

Patients affected by SBCAD deficiency present hypoglycemia, metabolic acidosis, mental retardation, seizures, lethargy, hypotonia and delayed motor development with muscular atrophy (Sass et al., 2008; Alfardan et al., 2010). However, many patients may be asymptomatic for long periods, even life long, and only manifest symptoms when under stress caused by intercurrent illness (Andresen et al., 2000; Alfardan et al., 2010). The neuroimaging findings evidence cortical damage, white and gray matter abnormalities, as well as global hypoxia (Gibson et al., 2000; Madsen et al., 2006; Sass et al., 2008).

Although SBCAD deficiency is characterized by neurological symptoms, the pathogenesis of brain damage of this disease is virtually unknown. It has been suggested that the accumulating metabolites (2MBG and 2MB) are potentially neurotoxic, especially during crises of metabolic decompensation, where the concentrations of these compounds increase dramatically (Andresen et al., 2000; Sweetman and Williams, 2001; Sass et al., 2008). However, the mechanisms by which these compounds could lead to the neurological symptoms and abnormalities characteristic of this disorder have not yet been elucidated. Therefore, in the present study we investigated the *in vitro* effects of 2MBG and 2MB on important parameters of oxidative stress, namely thiobarbituric acid-reactive substances (TBA-RS) levels, carbonyl formation, sulfhydryl oxidation, glutathione (GSH) levels and nitric oxide production in cerebral cortex of young rats. We also evaluated the effects of 2MBG on GSH concentrations and cell viability and integrity in cultured C6 glioma cells.

2. Results

2.1. 2MBG induces lipid peroxidation

First, we investigated the *in vitro* effects of 2MBG and 2MB on TBA-RS levels in cerebral cortex of 30-day-old rats. Fig. 1 shows that 2MBG [$F_{(5,24)}=38.237$; $P<0.001$], but not 2MB, significantly increased TBA-RS levels in a dose-dependent manner [$\beta=0.918$; $P<0.001$] at 0.5 mM and higher concentrations (up to 55%) in brain supernatants. We then evaluated the role of antioxidants on 2MBG-induced lipid peroxidation. Cortical supernatants were co-incubated with the antioxidants MEL (750 μM), GSH (750 μM) or TRO (α-tocopherol; 7.5 μM) and 5.0 mM 2MBG. Our results show that all antioxidants were able to fully prevent MBG-induced increase of TBA-RS levels (MEL: [$F_{(3,20)}=21.674$; $P<0.001$]; GSH: [$F_{(3,20)}=20.632$; $P<0.001$]; TRO: [$F_{(4,25)}=31.777$; $P<0.001$]) (Fig. 2).

2.2. 2MBG and 2MB do not cause protein oxidation

We also observed that 2MBG and 2MB did not significantly increase carbonyl formation (Fig. 3), implying that protein oxidative damage was not elicited by these compounds.

2.3. 2MBG diminishes nonenzymatic antioxidant defenses

Then, the nonenzymatic antioxidant defenses were examined by assessing GSH levels and sulfhydryl oxidation in brain cortical supernatants. Fig. 4A shows that 2MBG, but not 2MB (Fig. 4B), significantly diminished GSH levels in cerebral cortex supernatants (up to 38%) [$F_{(5,24)}=15.589$; $P<0.001$] in a dose-dependent manner [$\beta=-0.832$; $P<0.001$]. We also found that GSH levels were not changed by 2MBG (0.1–5 mM) when cortical supernatants were heated at 60 °C for 1 h, implying that 2MBG-induced GSH oxidation was probably dependent on protein factors that were probably denatured by heating (Fig. 4C). Finally, we verified that 2MBG decreased GSH concentrations (43%) in C6 glioma cells after 1 h of exposure [$t_{(4)}=3.271$; $P<0.05$] (Fig. 4D).

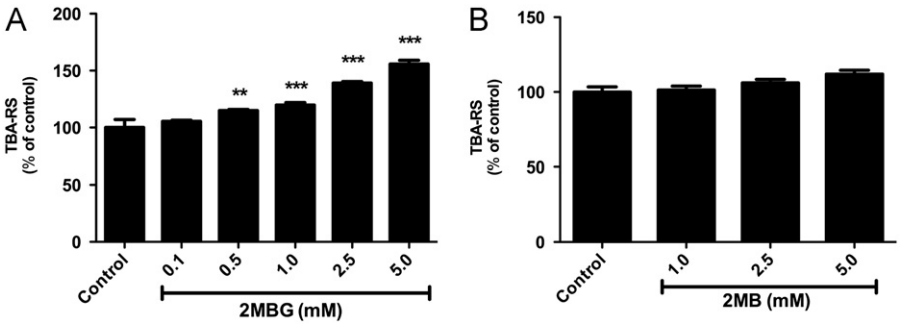

Fig. 1 – *In vitro* effects of 2-methylbutyrylglycine (2MBG) (A) and 2-methylbutyric acid (2MB) (B) on thiobarbituric acid-reactive substances (TBA-RS) in rat cerebral cortex. Values are means ± standard deviation for six independent experiments performed in triplicate and expressed as percentage of controls (Controls [nmol/mg protein] (A) 1.18 ± 0.19; (B) 1.09 ± 0.06). $^{**}P<0.01$, $^{***}P<0.001$, compared to controls (Duncan multiple range test).