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Taurine supplementation does not decrease homocysteine levels and liver injury induced by a choline-deficient diet

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ABSTRACT

Aims: The aim of this study is to examine the effects of taurine supplementation on homocysteine (Hcy) metabolism and liver injury in rats fed a choline-deficient diet.
Main methods: Thirty rats were divided into three groups ($n = 10$), to receive one of the following diets for 4 weeks: control diet (C), choline-deficient diet (CDD), or choline-deficient diet supplemented with taurine (CDDT). The CDD and the CDDT consisted of AIN-93 without the recommended choline content of 2.5%, and the CDDT was supplemented by the addition of 2.5% taurine.
Key findings: Four weeks of ingesting a CDD resulted in a significant increase in plasma Hcy (50%) as well as a decrease in liver S-adenosylmethionine (SAM) concentration and S-adenosylmethionine/S-adenosylhomocysteine ratio. No changes were found in plasma methionine and cysteine plasma levels compared to control group. Four weeks of ingesting a CDD also caused a significant ($P < 0.05$) increase in hepatic total fat, hepatic malondialdehyde (MDA), and plasma alanine aminotransferase (ALT) levels. In addition, reduced hepatic glutathione (GSH) levels and reduced/oxidized glutathione ratios (GSH/GSSG) were found in rats fed a CDD compared to controls. Taurine supplementation of the CDD normalized genes involved in the remethylation pathway, *BHMT* and *CHDH*, which were impaired by CDD alone. However, taurine supplementation failed to prevent CDD-induced Hcy metabolism disturbances and hepatic injury. Also, taurine added to CDD caused decreased expression of *PEMT*, *CHKa*, and *CHKb*, key genes involved in phosphatidylcholine (PC) synthesis and liver fat accumulation.
Significance: Taurine supplementation failed to ameliorate impaired Hcy metabolism and liver injury caused by CDD intake.

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Introduction

In the last few years, elevated blood concentration of homocysteine (Hcy) has been considered a risk factor for the development of cardiovascular disease (Steed and Tyagi, 2011). Numerous studies have also related hyperhomocysteinemia (HHcy) to several diseases, such as neurodegenerative disease, diabetes, renal dysfunction, fatty liver disease and others (Oulhaj et al., 2010; Wijekoon et al., 2005; Taes et al., 2004). Hcy is an amino acid formed exclusively by demethylation of methionine (Deminice et al., 2013). In Hcy synthesis, S-adenosylmethionine (SAM) acts primarily as a universal methyl donor in the synthesis of methylated compounds. Once formed, Hcy can be remethylated to form methionine by two parallel pathways, both of which may lower Hcy concentrations. The betaine-homocysteine S-methyltransferase (BHMT) remethylation pathway requires betaine, which is the methyl donor in this reaction and is derived from dietary choline (Deminice et al., 2013; Brosnan et al., 2007). Studies have shown that a choline-deficient diet (CDD) impairs methionine remethylation, increases Hcy levels (Setoue et al., 2008) and promotes liver injury characterized by oxidative stress and fatty liver (Raubenheimer et al., 2006; Ueland, 2011).

As an end product of the transsulfuration pathway in Hcy metabolism, taurine supplementation may play a crucial role in methionine metabolism in the liver (Fig. 1). Taurine (2-minoethanesulfonic acid) is a sulfur-containing amino acid synthesized endogenously from cysteine or obtained by dietary components, especially fish and seafood (Stipanuk and Ueki, 2011). Recently, taurine supplementation has shown protective effects—such as antioxidant properties—to improve insulin sensitivity and dyslipidemia, as well as fatty liver disease, in rat models of obesity, diabetes type 2, and steatohepatitis (Balkan et al., 2002; Rosa et al., 2014; Xiao et al., 2008). However, the effects of taurine supplementation on Hcy metabolism disturbances and liver injury,

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especially those induced by CDD, have been poorly explored. Recently, Yang et al. (2009) demonstrated that taurine supplementation decreased Hcy through improved utilization of sulfur-containing amino acids after alcohol administration. We hypothesized that taurine supplementation may modulate the catabolism of Hcy through the transsulfuration pathway and alleviate disturbances in methionine metabolism caused by CDD intake. The aim of the present study was to examine the effects of taurine supplementation on Hcy metabolism and hepatic injury in rats fed a CDD.

Methods

Rats and treatment

Thirty male Wistar rats (initial weight ~140 g) were obtained from the Faculty of Medicine of Ribeirao Preto Central Animal Care. All procedures were approved by the Ethics Committee for Animal Use of the same institution and were in accordance with the Guidelines of the COBEA (Brazilian College of experiments with animals). The rats were housed in individual cages on a 12-h-light/-dark cycle at a mean temperature of 22 °C and were randomly assigned to into three groups of 10 rats each: control (C); choline-deficient diet (CDD); choline-deficient diet with taurine supplementation (CDDT). C group was fed with AIN-93 standard diet proposed by Reeves et al. (1993) composed by 2.5% of choline. The CDD and the CDDT consisted of AIN-93 without the recommended choline content of 2.5%, and the CDDT was supplemented by the addition of 2.5% taurine. The rats had free access to food throughout the 4 weeks. Food intake was measured daily to assess food, choline and taurine consumption. Body weight was measured twice a week to determine the weight gain.

Tissue preparation

After four weeks of experiment, rats were anesthetized with an intra-peritoneal injection of sodium pentobarbital (65 mg/kg i.p.). Animals were sacrificed between 9 and 11 am. Blood was collected into heparinized tubes, centrifuged and the plasma stored at −80 °C. A portion of the liver was freeze-clamped with aluminum tongs pre-cooled in liquid nitrogen, weighed and stored at −80 °C. All procedures were performed under standard RNase-free conditions to avoid exogenous RNase contamination.

Homocysteine and metabolites

Plasma and liver Hcy levels and sulfur-containing amino acids were assayed by gas-chomatography (GC-FID, GC-17A Shimadzu®, Kyoto, Japan) derivatized with commercially available kit EZ:Faast Amino Acid Analysis (Phenomenex®). For SAM and SAH determinations, freeze-clamped liver samples were homogenized in ice-cold 8% (wt./vol.) trichloroacetic acid and the homogenates centrifuged at 13,000 g for 5 min at 4 °C. The supernatants were analyzed by HPLC (LC-20A Shimadzu®, Kyoto, Japan) using a Phenomenex® C18 column equilibrated with 96% of buffer A (50 mmol/L NaH_2PO_4 containing 10 mmol/L heptanesulfonic acid at pH 3.2) and 4% acetonitrile. SAM and SAH were separated by means of a gradient of 96–80% of *buffer A* and 4–20% of acetonitrile for 25 min. SAM and SAH peaks were detected at 258 nm and quantified using LC solution software (Shimadzu®, Kyoto, Japan).

Indices of hepatic injury

Plasma alanine aminotransferase (ALT) activity was performed using commercially available kit (Labtest, Lagoa Santa, Minas Gerais, Brazil). Liver malondialdehyde (MDA) was determined by HPLC (LC-20A Shimadzu®, Kyoto, Japan) according to Spirlandeli et al. (2014). Hepatic reduced (GSH) and oxidized glutathione (GSSG) were performed as described by Rahman et al. (2006). Liver total fat was determined by homogenizing 0.5 g of liver in 1.0 mL of distilled water; 5 mL of chloroform–methanol (2:1) was added and the tubes were thoroughly mixed. After centrifugation, the chloroform phase was transferred to a pre-weighed tube, the extraction was repeated twice and the chloroform phases were combined, evaporated to dryness and re-weighed. The fat was re-suspended in 1 mL 1-propanol for the measurement of total liver triglycerides, using commercially available kits from Labtest (Lagoa Santa, Minas Gerais Brazil).

Gene expression

Total RNA was isolated from 50 mg of frozen liver using RiboPure Kit (Ambion, part number AM 1924, USA) according to the manufacturer's instructions. Total RNA was quantified by spectrophotometer at OD 260/280 (NanoDrop2000c, USA). The quality and integrity of the isolated RNA were assessed using agarose gel (1.2%). Additional DNase I treatment (DNA-free Kit, Ambion, part number AM1906, USA) was performed to remove contaminating DNA from isolated total RNA. cDNA was synthesized from 1000 ηg of total RNA using High-capacity cDNA Reverse Transcription Kit (Applied Biosystems, part number 4374966, USA). Quantitative real-time PCR was performed using 7500 Fast Real-time PCR System (Applied Biosystems, USA). The following Taqman® Gene Expression Assays (Applied Biosystems, USA) were used in this study: Rn00564517_m1 (Pemt), Rn00578255_m1 (Bhmt), Rn00567215_m1 (Gnmt), Rn00560948_m1 (Cbs), Rn01644299_m1 (ChDh), Rn00567492_m1 (Chka), Rn00755199_g1 (Chkb), Rn00589584_m1 (Pcyt 1a), and Rn00690933_m1 (cyclophilin A). The cycles for PCR were as follows: one cycle of 95 °C for 20 s, 40 cycles of 30 s at 95 °C, 30 s at 60 °C. Each PCR assay was performed in triplicate. Cyclophilin A was used as reference gene to normalize the reactions. The relative quantitation was determined by the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Data were reported as means ± standard error. Groups were compared by ANOVA one-way and post-test of Tukey to identify specific differences between pairs of treatments using the SAS statistical package (version 8.2). In all analyses, the level of significance was set at $P < 0.05$.

Results

No differences in body weight gain, food intake, or liver weight were observed for the three groups during the 4-week experimental period. As expected, choline and taurine intake were higher ($P < 0.05$) in the C and CDDT groups, respectively, compared to the other groups (Table 1).

CDD intake resulted in a significant increase in plasma Hcy levels (50%) as well as decreased liver SAM concentration (25%) and decreased SAM/SAH ratio (34%) (Fig. 1). No changes were observed in plasma methionine and cysteine in the CDD group compared to the control group. Four weeks of CDD intake also exacerbated hepatic injury, as evidenced by a significant ($P < 0.05$) increase in hepatic total fat hepatic MDA and plasma ALT. In addition, reduced GSH and lower GSH/GSSG ratio were found in rats fed CDD compared to controls Table 2. Supplementation with taurine does not prevent Hcy metabolism disturbances and hepatic injury induced by CDD.

We examined the abundance of genes involved in the metabolism of Hcy and choline. CDD decreased the expression of genes involved in the remethylation pathway (*BHMT* and *CHDH*) compared to control group. Taurine supplementation reversed these perturbations. However, taurine added to CDD resulted in decreased phosphatidylethanolamine N-methyltransferase (*PEMT*) gene expression, as well as lower *CHKa* and *CHKb* gene expression. This result was not found in C or CDD-fed

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