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Choline cannot be replaced by propanolamine in mice

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Abstract

Choline is an important nutrient for humans and animals. Animals obtain choline from the diet and from the catabolism of phosphatidylcholine made by phosphatidylethanolamine *N*-methyltransferase (PEMT). The unique model of complete choline deprivation is *Pemt*$^{-/-}$ mice that are fed a choline-deficient diet. This model, therefore, can be used for the examination of choline substitutes in mammalian systems. Recently, propanolamine was found to be a replacement for choline in yeast. Thus, we tested to see whether or not choline can be replaced by propanolamine in mice. Mice were fed a choline-deficient diet and supplemented with either methionine, 2-amino-propanol, 2-amino-isopropanol and 3-amino-propanol. We were unable to detect the formation of any of the possible phosphatidylpropanolamines. Moreover, none of them prevented liver damage, reduction of hepatic phosphatidylcholine levels or fatty liver induced in choline-deficient-*Pemt*$^{-/-}$ mice. These results suggest that choline in mice cannot be replaced by any of the three propanolamine derivatives.

Keywords: Choline; Phosphatidylethanolamine *N*-methyltransferase; Phosphatidylcholine; Propanolamine; Choline-deficient

1. Introduction

Choline means bile alkaline, which is from the original finding of this compound in ox bile (the Greek word for bile is chole) in 1862 [1]. Choline is alkaline and hydrophilic. In 1932, Best and Huntsman first reported that choline was an important nutrient for mammals [2]. After intensive research on choline in the middle of the 20th century [3], the nutritional importance of choline has been investigated [4–8]. In 1998, a recommendation for daily intake of choline for humans was made by the 'Food and Nutrition Board' in the U.S. The recommended intake of choline for adults is ~500 mg/day (equal to ~1250 mg choline bitartrate a day). In 2001, the U.S. Food and Drug Administration (FDA) released "Nutrient Content Claims Notification for Choline Containing Foods" (www.fda.gov). At this time, choline was officially approved as a nutraceutical (nutrition+pharmaceutical) drug. In addition, several choline derivatives, such as citicoline (CDP-choline) [9,10] and betaine [11], were used in clinical therapy.

In animals, there are two choline acquisition pathways: dietary choline intake and the endogenous biosynthesis of choline through the methylation of phosphatidylethanolamine (PE) into phosphatidylcholine (PC) catalyzed by PE *N*-methyltransferase (PEMT) [12] and subsequent catabolism to choline. The latter is the only known endogenous pathway for choline biosynthesis in mice. Either exogenous or endogenous choline flows to PC, which is the major choline metabolite accounting for about 95% of total choline-containing metabolites [5,13]. PEMT is found in a significant amount only in the livers of animals and accounts for about 30% of hepatic PC biosynthesis [14–17]. The other 70% of hepatic PC is made via the CDP-choline pathway.

Mice that lack PEMT fed a choline-deficient (CD) diet are a unique model of complete choline deprivation, devoid of choline from both endogenous and exogenous sources [18,19]. CD–*Pemt*$^{-/-}$ mice died of acute liver failure and steatohepatitis within 4–5 days indicating that complete choline deprivation is lethal [18,19].

Abbreviations: CD, choline-deficient; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; ALT, alanine aminotransferases; TG, triacylglycerol; AdoMet, *S*-adenosylmethinonine

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Whether or not choline can be replaced by other compounds is an intriguing question. Initial studies were performed with dimethylethanolamine in place of choline in CD–*Pemt*$^{-/-}$ mice [20]. Previously it had been shown that dimethylethanolamine could be converted to phosphatidyldimethylethanolamine and that phosphatidyldimethylethanolamine had physical properties that were similar to PC [21,22]. However, dimethylethanolamine was not a good substitute for choline and the CD–*Pemt*$^{-/-}$ mice fed dimethylethanolamine only lived 1 day longer than CD–*Pemt*$^{-/-}$ mice [20]. Recently, Voelker's laboratory [23,24] found that choline could be replaced by propanolamine in yeast [23,24]. This led us to examine whether or not propanolamine and/or its derivatives could replace choline in mammalian systems. The experiments were performed with CD–*Pemt*$^{-/-}$ mice (the unique model of complete choline deprivation). In addition, we also examined if supplementation of methionine could rescue CD–*Pemt*$^{-/-}$ mice from liver failure.

2. Materials and methods

2.1. Animals

Pemt$^{-/-}$ mice (C57BL/6; 129/J background) [25] were fed a CD diet, a semi-synthetic diet without choline (ICN, Cat #0290138710), a choline-supplemented (CS) diet (a CD diet containing 0.4% (w/w) choline chloride), a propanolamine-supplemented diet (a CD diet containing 0.4% (w/w) 2-amino-propanol, 2-amino-isopropanol and 3-amino-propanol (Sigma)) or a methionine-supplemented diet (a CD diet containing 0.4% (w/w) methionine). At the age of 10 to 12 weeks, *Pemt*$^{-/-}$ mice were fed one of these diets for 3 days. Mice were fasted for 12 h before sacrifice. Three mice of each dietary group were used in all experiments and assays were performed in duplicate. All data are presented as means±S.D. *P* values are from comparisons to CD mice with a two-tail *t*-test.

2.2. Liver damage assays

Plasma samples were collected from *Pemt*$^{-/-}$ mice fed one of these diets for 3 days. Blood was collected by cardiac puncture with instruments pre-treated with EDTA. Plasma was separated by centrifugation at 2000 rpm for 20 min in a refrigerated bench-top centrifuge. Plasma alanine aminotransferase (ALT) activities were measured with a GPT/GOT Kit (*Sigma,* catalog #P505) as an indicator of liver damage.

2.3. Lipid analysis

Livers were frozen in liquid N_2 after dissection. All samples were stored at −70 °C before use. Livers were homogenized with a Polytron homogenizer in 5 vol 10 mM Tris–HCl, pH 7.2, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1:100 protease inhibitor cocktail (*Sigma*, Cat #P8340). After sonication, homogenates were centrifuged for 5 min at 600×*g* and supernatants collected. Protein was quantified [26] and total lipids were extracted from liver homogenates [27]. Phospholipids were separated by high-performance liquid chromatography and quantified with an electron-light scattering detector [28]. Phosphatidyldimethylethanolamine was used as an internal standard for quantification. Hepatic triacylglycerols (TG) were measured by gas–liquid chromatography [29]. To test whether phosphatidylpropanolamine was produced or not in our mouse models, phospholipids were separated by two-dimensional thin-layer chromatography on Silica 60 plates; first phase: chloroform/methanol/ammonium hydroxide (65:35:5, v/v/v); second phase: chloroform/acetic acid/methanol/water (75:25:5:2.2, v/v/v) [23,24,30]. Phosphatidylpropanolamine standard was kindly provided by Dr. Dennis Voelker.

3. Results

3.1. Phosphatidylpropanolamines are not detected in phospholipids from CD–*Pemt*$^{-/-}$ mice fed propanolamine derivatives

We examined three kinds of propanolamine derivatives, 2-amino-propanol, 2-amino-isopropanol and 3-amino-propanol as well as the CS and CD controls (Fig. 1A). By two-dimensional thin-layer chromatography, the phosphatidylpropanolamine standard was separated from PC and PE (Fig. 1B) [23,24]. However, we did not find any phosphatidylpropanolamine formed in CD–*Pemt*$^{-/-}$ mice fed any of the propanolamines (Fig. 1). Moreover, we did not detect phosphatidylpropanolamine by high-performance liquid chromatography when we quantified phospholipid compositions of the livers.

3.2. Propanolamine derivatives and methionine do not prevent liver damage induced in CD–*Pemt*$^{-/-}$ mice

Elevated plasma ALT activities are hallmarks of liver damage. Plasma ALT activities were assayed to evaluate the extent of liver damage. Liver damage in CD–*Pemt*$^{-/-}$ mice was found as previously observed [18,19]. However, none of three propanolamine derivatives prevented liver damage induced in CD–*Pemt*$^{-/-}$ mice (Fig. 2). In addition, an extra supply of methionine in the diet did not prevent liver damage induced in CD–*Pemt*$^{-/-}$ mice (Fig. 2) since these mice could not make PC from PE and *S*-adenosylmethionine (AdoMet).

3.3. Propanolamine derivatives and methionine do not prevent the reduction of hepatic PC levels and PC/PE ratios in CD–*Pemt*$^{-/-}$ mice

As we observed previously [18,19], hepatic PC levels in CD–*Pemt*$^{-/-}$ mice decreased ~50% compared to CS–*Pemt*$^{-/-}$ mice. Dietary supplementation of one of the three propanolamine derivatives or methionine did not prevent the ~50% reduction of hepatic PC levels compared to CS–*Pemt*$^{-/-}$ mice, and 2- or 3-aminopropanol supplementation caused a more severe reduction of hepatic PC levels compared to the CD diet (Fig. 3A). However, hepatic PE levels were not affected significantly by either propanolamines or methionine in CD–*Pemt*$^{-/-}$ mice (Fig. 3B). Thus, hepatic PC/PE ratios in these mice were also decreased to a similar extent as observed in CD–*Pemt*$^{-/-}$ mice compared to CS–*Pemt*$^{-/-}$ mice (Fig. 3C). Interestingly, 2- or 3-aminopropanol resulted in more reduction of PC/PE ratios compared to CD groups (Fig. 3C).

3.4. Propanolamine derivatives and methionine do not prevent steatosis induced in CD–*Pemt*$^{-/-}$ mice

The CD diet induced not only liver damage, but also fatty liver in *Pemt*$^{-/-}$ mice [18,19]. The CD diet caused an ~5 fold accumulation of TG in the livers of *Pemt*$^{-/-}$ mice compared to CS mice (Fig. 4). This was consistent with our previous findings [31]. None of three propanolamine derivatives nor methionine