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Taurine alleviates dyslipidemia and liver damage induced by a high-fat/cholesterol-dietary habit

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ABSTRACT

Eight male hamsters per group were assigned randomly to one of the following diets: chow diet (Control); high-fat/cholesterol diet (HFCD); HFCD supplemented with 1% Tau (HFCD/1% Tau); HFCD supplemented with 2% Tau (HFCD/2% Tau). Tau supplementation improved ($P < 0.05$) serum lipids and cholesterol profile in the high-fat/cholesterol-dietary hamsters. Although hepatic cholesterol/triacylglycerol in the high-fat/cholesterol-dietary hamsters were not ($P > 0.05$) changed by Tau supplementation, faecal cholesterol and bile acid outputs were increased ($P < 0.05$). Two percent Tau supplementation unregulated ($P < 0.05$) HMG-CoA reductase and cholesterol 7-α hydroxylase (CYP7A1) expressions in the high-fat/cholesterol-dietary hamsters. Besides, Tau supplementation also increased ($P < 0.05$) LDL receptor mRNA expressions in high-fat/cholesterol-dietary hamsters. Tau supplementation also reduced serum GPT and GOT values and C-reactive protein (CRP) levels in the high-fat/cholesterol-dietary hamsters. Results clearly indicated that Tau could alleviate blood lipids and hepatic damage induced by a high-fat/cholesterol-dietary diet.

1. Introduction

Lifestyle-related disorders, namely obesity, diabetes, hyperlipidemia and hypertension, are threatening human health and are regarded as important risks in the development of cardiovascular disease (CVD). CVD has been the number one leading cause of human death in the United States (Lloyd-Jones et al., 2009) as has also been observed in Taiwan. Cerebrovascular disease, heart disease, and hypertensive disease are second, third, and tenth major causes of death in Taiwan, respectively (Department of Health, Executive Yuan, ROC, 2008). The common epidemic reason for hyperlipidemia is excessive or improper lipid intake.

Taurine (Tau) (2-amino ethanesulphonic acid) is one of the major and free intracellular amino acids in many mammalian tissues, such as brain, retina, myocardium, skeletal muscle, liver, platelets, and leukocytes (Lee et al., 2004). Its bio-physiological functions include detoxification, antioxidant, membrane stabilization and osmoregulation, neuromodulation, and brain and retina development (Lee et al., 2004). Tau is only considered in lipid metabolism when it conjugates with bile acids in the liver, which increases the use of bile acids that emulsify dietary fat to form micelles for fat absorption in the small intestine (Yamanaka, Tsuji, & Ichikawa, 1986). Serum cholesterol (TC) and triacylglycerol (TAG), as well as serum cholesterol profile (the ratio of high-density lipoprotein cholesterol/total cholesterol, HDL-C/TC) are known as biomarkers for CVD. For decreasing risk of CVD, scientists try to decrease TC and TAG, or to increase HDL-C/TC. Zhang et al. (2004) recruited

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two groups of overweight or obese non-diabetic volunteers (Tau supplementation group, $n = 15$; placebo group, $n = 10$) in investigating the anti-atherogenic effect of Tau. Their clinical data [TG, TC, HDL-D, atherogenic index (AI)] indicated that Tau had an anti-atherogenic effect. However, the hypolipidemic mechanism of Tau is still unclear.

As we know, serum cholesterol homeostasis is regulated via cholesterol biosynthesis, catabolism and output, and serum cholesterol clearance. 3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) is the control point for cholesterol biosynthesis (Kritchevsky, 1987) and cholesterol 7-α hydroxylase (CYP7A1) is the rate-limiting enzyme for cholesterol degradation to bile acids (Zhang, Ho, Huang, & Chen, 2002), while the LDL receptor also plays an important role in clearance of cholesterol from blood (Dietschy, 1998). Hence, the relationship of HMG-CoA reductase, CYP7A1 and LDL receptor gene expressions are often discussed in hypolipidemic studies. Most likely, Golden Syrian hamsters are regarded as the best animal model to study the hypolipidemic effect, rather than rats or mice, because their blood lipid profile is extremely similar to human (Lehmann, Bhargava, & Gunzel, 1993). Therefore, via a high-fat/cholesterol-dietary-hamster model, the effects of Tau supplementation on serum lipids and enzymes related to cholesterol metabolism were investigated in the present study. A high-fat/cholesterol-dietary habit may induce hepatic damage, such as fatty liver; therefore, we also examined whether Tau could amend liver damage from a high-fat/cholesterol-dietary habit.

2. Materials and methods

2.1. Animals and diets

The animal use and protocol were reviewed and approved by Chung Shan Medical University Animal Care Committee. Thirty-two male Golden Syrian hamsters (5 weeks old) were purchased from the National Laboratory Animal Center (National Science Council, Taipei, Taiwan). Two hamsters were housed in each cage in an animal room at 22 ± 2 °C with a 12/12 h light–dark cycle. Chow diets containing 48.7% (w/w) carbohydrate, 23.9% (w/w) protein, 5.0% (w/w) fat, 5.1% (w/w) fiber, and 7.0% ash (Laboratory Rodent Diet 5001, PMI® Nutrition International/Purina Mills LLC., USA) and water were provided for one week of acclimatisation. Hamsters were randomly divided into four groups (Control, HFCD, HFCD/1% Tau; HFCD/2% Tau), two hamsters per cage. The Control group received the chow diet. 7% (w/w) soybean oil/0.1% (w/w) cholesterol into 92.9%, 91.9%, and 90.9% (w/w) chow diet, supplemented with and 0%, 1%, and 2% Tau, respectively, was assigned to the HFCD groups.

Soybean oil was purchased from Taiwan Sugar Co. (Taiwan). Cholesterol was purchased from ICN Biomedicals Inc. (Irvine, CA, USA). Taurine was obtained from Forever Chemical Co. Ltd. (Taiwan) with a purity of 99.6%. All hamsters were fed assigned diets and water *ad libitum*. The experimental period lasted for 6 weeks. The body weight of hamsters was individually recorded every three weeks. Feed and water were changed three times per week. Daily feed (g) and water intakes (ml) were calculated on a per hamster per day basis every 3 weeks.

2.2. Collection of serum, liver, visceral fat, and faeces

Blood samples were collected via puncturing the retroorbital sinus by a capillary tube, after a 16-h fast, at weeks 0 and 3. At the end of the experiment (week 6), all feed was removed 16 h before killing. All hamsters were euthanized by carbon dioxide. Liver and visceral fat from each hamster were collected and weighed. Liver was stored at −80 °C for further analyses. Blood samples were also collected by intracardiac puncture. Serum was separated from blood samples by centrifugation 3000 × *g* for 10 min, and then stored at −80 °C for further analyses. Faeces per cage were collected 72 h in advance of the end of experimental period and stored at −80 °C for further analyses.

2.3. Determination of serum lipid parameters

Serum total cholesterol (TC), triacylglycerol (TAG), and high-lipoprotein cholesterol (HDL-C) were measured by using commercial kits (Randox Laboratories Ltd., Antrim, UK). In the HDL-C analysis, low-density-lipoprotein (LDL), very-low-density-lipoprotein (VLDL), and chylomicron in serum were precipitated by addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation (3000 × *g* for 10 min), the cholesterol concentration in the HDL fraction was determined by using the TC commercial kit (Randox Laboratories Ltd., Antrim, UK). Those methods are based on detection of coloured end-products at 500 nm. The cardiac index (CI) was calculated by the formulation of TC/HDL-C (Kang, Lee, & Lee, 2004).

2.4. Determination of hepatic and faecal cholesterol and triacylglycerol and faecal bile acid

Hepatic and oven-dried faecal lipid extractions were measured according to the procedures of (Tzang et al., 2009). Briefly, hepatic and dried faecal lipids were extracted by chloroform and methanol (2:1, v/v). The extract was dried under N_2 and resuspended in isopropanol. The hepatic and faecal cholesterol and triacylglycerol concentrations were measured by using commercial kits (Randox Laboratories Ltd., Antrim, UK). 3α-Hydroxy bile acids comprise about 60% of the faecal bile acids in the hamster (Daggy, O'Connell, Jerdack & Stinson, 1997). and 3α-hydroxy bile acids can be determined using an enzymatic method (Randox Laboratories Ltd., Antrim, UK) (Trautwein, Rieckhoff, & Erbersdobler, 1998).

2.5. Hepatic mRNA expressions of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), low-density lipoprotein receptor (LDL receptor), cholesterol 7-α hydroxylase (CYP7A1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Total RNA was isolated from the stored frozen liver tissues by using the protocol described by RNeasy Mini Kits (Qiagen, Valencia, CA). Reverse transcription was carried out with 2 μg of total RNA, 8 μl of reaction buffer, 2 μl of dNTPs, 4.8 μl of $MgCl_2$, 4 μl of Oligo-dT (10 pmol/l) and 200 U of RTase (Promega, Madison, WI, USA) with DEPC H_2O in a final volume of 40 l at 42 °C for 1 h. After a heat inactivation, 1 μl of cDNA product was used for a PCR amplification. The appropriate primers of target genes were designed for hamster's HMG-CoA reductase, LDL receptor, CYP7A1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as follows (Tzang et al., 2009): HMG-CoA reductase sense 5′-AACTGAGAG CACAAGCAGAG-3′, antisense 5′-ATCACAAGCACGAGGAAGAC-3′; LDL receptor sense 5′-ACAGATTCAGTTCCAGGCAG-3′, antisense 5′-TGGGGACAAGAGGTTTTCAG-3′; CYP7A1 sense 5′-TTTGGACAC AGAAGCATT-3′, antisense 5'-TCCATGTCATCAAAGGTA-3′; GAPDH sense 5′-GACCCCTTCATTGACCTCAAC-3′, antisense 5′-GGAGAT-GATGACCCTTTTGGC-3′. The size of reaction products is as follows: for HMG-CoA reductase, 583 bp; LDL receptor, 477 bp; CYP7A1, 497 bp; GAPDH, 264 bp. GAPDH was used as an internal control in all reactions. The PCR amplification was performed using a DNA thermal cycler (ASTEC PC-818, ASTEC Co., Ltd., Fukuka, Japan) under the following conditions: HMG-CoA reductase: 30 cycles for 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min, followed by 10 min at 72 °C; LDL receptor and CYP7A1: 30 cycles for 94 °C for