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The pathophysiological role of oxidized cholesterols in epicardial fat accumulation and cardiac dysfunction: a study in swine fed a high caloric diet with an inhibitor of intestinal cholesterol absorption, ezetimibe

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Abstract

Oxidized cholesterols (oxycholesterols) in food have been recognized as strong atherogenic components, but their tissue distributions and roles in cardiovascular diseases remain unclear. To investigate whether accumulation of oxycholesterols is linked to cardiac morphology and function, and whether reduction of oxycholesterols can improve cardiac performance, domestic male swine were randomized to a control diet (C), high caloric diet (HCD) or HCD + Ezetimibe, an inhibitor of intestinal cholesterol absorption, group (HCD + E) and evaluated for: (1) distribution of oxycholesterol components in serum and tissues, (2) levels of oxycholesterol-related enzymes, (3) paracardial and epicardial coronary fat thickness, and (4) cardiac performance. Ezetimibe treatment for 8 weeks attenuated increases in oxycholesterols in the HCD group almost completely in liver, but reduced only levels of 4β-hydroxycholesterol in left ventricular (LV) myocardium. Ezetimibe treatment altered the expression of genes for cholesterol and fatty acid metabolism and decreased the expression of CYP3A46, which catabolizes cholesterol to 4β-hydroxycholesterol, strongly in liver. An increase in epicardial fat thickness and impaired cardiac performance in the HCD group were improved by ezetimibe treatment, and the improvement was closely related to the reduction in levels of 4β-hydroxycholesterol in LV myocardium. In conclusion, an increase in oxycholesterols in the HCD group was closely related to cardiac hypertrophy and dysfunction, as well as an increase in epicardial fat thickness. Ezetimibe may directly reduce oxycholesterol in liver and LV myocardium, and improve cardiac morphology and function.

Keywords: Obesity; Epicardial fat; Oxycholesterol; Cardiac function; Cardiac hypertrophy

1. Introduction

Total and low-density lipoprotein (LDL) cholesterol are considered important risk factors for cardiovascular diseases. However, oxidized cholesterols (oxycholesterols), defined as oxygenated derivatives of cholesterol, are now thought to contribute to the development of cardiovascular diseases more than nonoxidized cholesterol [1,2]. In fact, the levels of major oxycholesterol components of oxidized LDL, such as 7-ketocholesterol and 7β-hydroxycholesterol, have been shown to be increased in the atherosclerotic lesions and plasma of patients with cardiovascular diseases [3]. Oxycholesterol is produced during food processing, when fats and oxygen meet and react

Abbreviations: ABCA1, ATP-binding cassette transporter 1; FAS, fatty acid synthase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LXR and LXRα, liver X receptor and liver X receptor α; LV, left ventricular; LVEF, left ventricular ejection fraction; NPC1L1, Niemann–Pick C1L1; oxycholesterol, oxidized cholesterol; PPARγ$_2$, peroxisome proliferator-activated receptor γ$_2$; RT-PCR, reverse transcription polymerase chain reaction; SAT, VAT and EAT, subcutaneous, visceral and epicardial adipose tissue; SREBP2 and SREBP1c, sterol regulatory element-binding protein 2 and 1c.

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chemically (auto-oxidation), but is also produced in the body by enzymatic oxidation [4]. Oxycholesterols, short-lived intermediates or end products of the catabolism or excretion of cholesterol, are present only at trace levels in biological membranes and lipoproteins, but can exert profound biological effects at these low concentrations [3,4]. For technical reasons, the tissue distributions and pathophysiological roles of oxycholesterol components have not yet been completely evaluated [3,4].

It has been shown that ezetimibe, which selectively blocks the uptake of biliary and dietary cholesterol *via* the Niemann–Pick C1L1 (NPC1L1) protein in the small intestine, reduces plasma levels of oxycholesterols in subjects with coronary artery disease [5] or hypercholesterolemia [6,7]. Alternatively, the addition of ezetimibe to a weight loss diet can decrease hepatic steatosis and visceral fat in subjects with obesity [8] or nonalcoholic fatty liver disease [9]. The accumulation of oxycholesterols [10] and reactive oxygen species [11] in adipose tissues is linked to obesity-associated metabolic derangement, and it is speculated that oxycholesterols play an active role in adipose dysfunction and the development of insulin resistance/type 2 diabetes [12]. Previously, we reported that the Zucker diabetic fatty rat model of obesity showed evidence of cardiac steatosis through the accumulation of saturated fatty acids and cardiac dysfunction [13]. Hence, we proposed that ectopic fat deposition in heart components, including circulatory and locally recruited fat, intra- and extra-myocellular fat, perivascular fat and pericardial and epicardial fat, could be closely related to cardiac dysfunction and cardiovascular complications (cardiac lipotoxicity) [14].

Based on the above, we hypothesized that the accumulation of oxycholesterols was linked to cardiac metabolism and function, and that reduction of oxycholesterol levels could improve cardiac performance. To study the link between oxycholesterols and cardiac fat and function, we evaluated the effects of ezetimibe on the distributions of oxycholesterol components in serum and tissues, the levels of oxycholesterol-related enzymes, paracardial and epicardial coronary fat thickness, and cardiac performance in high-oxycholesterol swine.

2. Materials and methods

2.1. Animals and treatment

A total of 12 domestic male swine weighing 21–26 kg (average 24.5 ± 1.4 kg) were randomized to receive either a control diet (C), a high caloric diet (HCD) or an HCD + Ezetimibe (HCD + E). The swine were housed individually in metabolic cages and fed a control diet (80 kcal/kg/day) containing 16% of calories from fat, or an HCD (120 kcal/kg/day) containing 16% of calories from fat and 1.5% cholesterol, or an HCD (120 kcal/kg/day) containing 16% of calories from fat and 1.5% cholesterol with oral administration of ezetimibe (10 mg/day) for 8 weeks. Body weight, food intake and blood pressure were monitored at baseline and after 4 and 8 weeks of treatment. Fasting blood samples were obtained through surgically implanted jugular vein catheters from unconscious animals before and after 8 weeks of treatment. Animal experiments were approved by the Committee on Animal Research of the University of Tokushima and conducted in accordance with international ethical principles and guidelines for experiments on animals.

2.2. Echocardiography

Transthoracic echocardiography was performed with the animal in the left lateral decubitus position under general anesthesia. We used vivid i ultrasound diagnostic system (GE Healthcare, Milwaukee, WI, USA) with 12 L-RS transducer (6.0–13.0 MHz Linear transducer) for the observation of epicardial and paracardial fat and 7S-RS transducer (3.5–8.0 MHz Sector transducer) for the other recording. End-diastolic and end-systolic left ventricular (LV) dimensions and thickness of interventricular septal and posterior wall were measured from M-mode echocardiogram of left ventricle at the papillary muscle level. Relative wall thickness was calculated as the ratio of LV posterior wall thickness to LV end-diastolic dimension. LV end-diastolic and end-systolic volume was calculated using method of disks from apical four- and two-chamber views of left ventricle. We also recorded transmitral flow velocity pattern from the apical long-axis view and obtained peak early diastolic (E) and atrial systolic (A) velocities, and the ratio of E to A (E/A) was calculated. Paracardial fat thickness was measured above the right ventricular free wall and epicardial fat thickness was measured in the anterior interventricular groove near coronary artery [15].

2.3. Sampling and biochemical assays

Swine were euthanized approximately 22 h after the last dose of ezetimibe, and tissue samples from liver, subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), epicardial adipose tissue (EAT) and LV myocardium were flash frozen and stored at −80 °C. The lipid and lipoprotein analysis methods used in this study have been described previously [11,13,16]. The lipoprotein distributions of cholesterol and triglycerides were evaluated in serum samples (50 μL) resolved by size exclusion chromatography on a Superose 6 column.

2.4. Oxycholesterol assays

Oxycholesterol levels were measured as previously described [16]. Briefly, 50 μg of 5α-cholestane (Sigma, St. Louis, MO, USA) and 1 μg of 19-hydroxycholesterol (Steraloids, Newport, RI, USA) were added to 0.25 g of liver and LV myocardium, and 100 μL of serum (internal standard). The lipids in the sample were extracted with 20 vol of chloroform/methanol (2:1, vol/vol) containing 0.01% (wt/vol) butylated hydroxy-toluene (Nacalai Tesque, Kyoto, Japan). The extracted lipids were applied to a Sep.-Pak Silica Vac cartridge (Nihon Waters, Tokyo, Japan) to separate oxycholesterols from sterols. The cartridge was sequentially eluted with 1 ml of hexane, 8 ml of a solvent mixture composed of hexane and 2-propanol (1:200, vol/vol), and 5 ml of a solvent mixture composed of hexane and 2-propanol (3:10, vol/vol), which allowed for sequential elution of 5α-cholestane, cholesterol plus phytosterols and 19-hydroxycholesterol plus oxysterols, respectively. The samples were allowed to saponify at room temperature in the dark overnight, and unsaponified lipids were converted into trimethylsilyl ethers. The gas chromatography (GC) analysis of sterols was performed as previously described [16]. GC–mass spectrometry was performed on a Shimazu GC-17 A version 3 coupled with a SPB-1 fused silica capillary column connected to a Shimadzu QP5050A series mass-selective detector. The variables of ions monitored, relative retention times, correlation coefficients for calibration curves, response factors for the monitored ions, detection limits and %CVs for repeated injection were determined as previously described [16].

2.5. Semiquantitative reverse transcription polymerase chain reaction analysis

After extracting total RNA from liver, SAT, VAT, EAT and LV myocardium samples, we synthesized cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA), and then performed reverse transcription polymerase chain reaction (RT-PCR) with gene-specific primers and SYBR green dye using an Applied Biosystems 7500 RT-PCR System (Life Technologies Japan Ltd., Tokyo, Japan). The primer and probe sets used for quantitative RT-PCR analysis are shown in Supplemental Table in the online version at http://dx.doi.org/10.1016/j.jnutbio.2016.05.010. Primers were purchased from Takara Bio Inc. (Kyoto, Japan). Values are expressed in arbitrary units after normalization for β-actin expression. Data were quantified using the ΔΔCT method [17].

2.6. Statistical analysis

Values are expressed as means (S.E.). One-way analysis of variance followed by Holm–Sidak multiple comparisons test was performed to compare group means. Pearson's linear regression test was used to identify significant associations of 4β-hydroxycholesterol in LV myocardium to echocardiographic findings. All statistical analyses were performed by GraphPad Prism version 6.0f for Mac OS10, GraphPad Software, La Jolla, CA, USA. A P value <.05 was considered statistically significant.

3. Results

3.1. General characteristics

As shown in Fig. 1 and Table 1, baseline body weight was comparable among the C, HCD and HCD + E groups. There were no statistical differences in the changes in body weight and daily calorie intake among three studied groups. Systolic and diastolic blood pressure and heart rate did not change in any group. Heart weight/body weight was comparable among the three groups.

3.2. Lipoprotein measurements

A borderline increase in the serum levels of cholesterol and phospholipids and in the myocardial levels of triglycerides was observed, in addition to an increase in liver levels of cholesterol