Atherosclerosis 242 (2015) 77–86

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Plasma cholesterol-lowering activity of dietary dihydrocholesterol in hypercholesterolemia hamsters

Xiaobo Wang [a], Lei Guan [b], Youyou Zhao [b], Lin Lei [a], Yuwei Liu [a], Ka Ying Ma [a], Lijun Wang [a], Sun Wa Man [a], Junkuan Wang [b], Yu Huang [c], Zhen-Yu Chen [a, *]

[a] Food & Nutritional Sciences Programme, School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China
[b] R&D, Nestle, Beijing 100022, China
[c] School of Biomedical Sciences, Chinese University of Hong Kong, Shatin, NT, Hong Kong, China

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form
19 June 2015
Accepted 22 June 2015
Available online 27 June 2015

Keywords:
5α-cholestanol
Cholesterol
β-sitosterol
Sterol

ABSTRACT

Objective: Cholesterol analogs have been used to treat hypercholesterolemia. The present study was to examine the effect of dihydrocholesterol (DC) on plasma total cholesterol (TC) compared with that of β-sitosterol (SI) in hamsters fed a high cholesterol diet.
Methods and Results: Forty-five male hamsters were randomly divided into 6 groups, fed either a non-cholesterol diet (NCD) or one of five high-cholesterol diets without addition of DC and SI (HCD) or with addition of 0.2% DC (DA), 0.3% DC (DB), 0.2% SI (SA), and 0.3% SI (SB), respectively, for 6 weeks. Results showed that DC added into diet at a dose of 0.2% could reduce plasma TC by 21%, comparable to that of SI (19%). At a higher dose of 0.3%, DC reduced plasma TC by 15%, less effective than SI (32%). Both DC and SI could increase the excretion of fecal sterols, however, DC was more effective in increasing the excretion of neutral sterols but it was less effective in increasing the excretion of acidic sterols compared with SI. Results on the incorporation of sterols in micellar solutions clearly demonstrated both DC and SI could displace the cholesterol from micelles with the former being more effective than the latter.
Conclusion: DC was equally effective in reducing plasma cholesterol as SI at a low dose. Plasma TC-lowering activity of DC was mediated by inhibiting the cholesterol absorption and increasing the fecal sterol excretion.

1. Introduction

Coronary heart disease (CHD) is the number one killer in the world. Elevated concentrations of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are considered as the major risk factors for CHD. One of pharmaceutical approaches to reduce plasma TC and LDL-C concentrations is to inhibit the cholesterol absorption in the intestine. In this regard, about 1200 mg cholesterol daily enters the lumen of the small intestine with 300 mg coming from the diet and the rest deriving from bile [1,2]. Absorption of sterols in the intestine is a function of their structures. In general, absorption of cholesterol can reach more than 50%, while that of plant sterols is less than 5% [3–5]. Plant sterols are analogs of cholesterol and have side chains different from that of cholesterol. Due to their poor absorption and structural similarity with cholesterol, plant sterols as a health supplement are very effective in reducing plasma TC and LDL-C, mediated by their strong inhibition on cholesterol absorption in the intestine [6]. It has been suggested to take 2 g plant sterols daily as a therapeutic option to lower TC and LDL-C by 6–15% in hypercholesterolemia patients [7].

Dihydrocholesterol (DC), also called 5α-cholestanol, is a cholesterol analog. DC has a same side chain as cholesterol, but it has no double bond at the Δ^5 position in B-ring (Fig. 1). Natural DC can be produced at least by the following three routes. First,

Abbreviations: ABCG5/8, ATP-binding cassette transporters sub-family G member 5 and 8; ACAT2, acyl-CoA: cholesterol acyltransferase 2; CYP7A1, cholesterol-7α-hydroxylase; DC, dihydrocholesterol; HDL-C, high density lipoprotein cholesterol; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; LDLR, low-density lipoprotein receptor; LXRα, Liver × receptor alpha; MTP, microsomal triacylglycerol transport protein; non-HDL-C, non-high density lipoprotein cholesterol; NPC1L1, Niemann-Pick C1 like 1 protein; SI, β-sitosterol; SREBP2, sterol regulatory element-binding protein 2; TC, total cholesterol; TG, triacylglycerols.

* Corresponding author. School of life Sciences, Chinese University of Hong Kong, Hong Kong, China.
E-mail address: zhenyuchen@cuhk.edu.hk (Z.-Y. Chen).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.06.046
0021-9150/

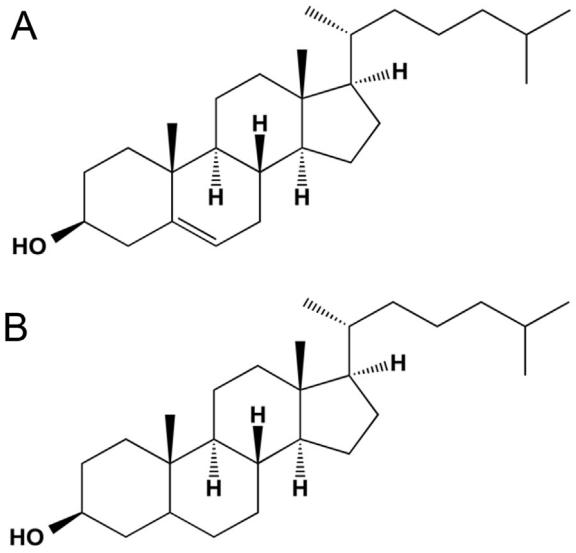

Fig. 1. Structures of cholesterol (A) and dihydrocholesterol (B).

unabsorbed cholesterol in the lumen of large intestine is biohydrogenated to form DC and coprostanol via the action of microbial enzymes [8]. Second, in environment, particularly in the anaerobic reducing sediments, bacteria can convert some cholesterol to DC [9]. Third, DC is synthesized in vivo via a pathway with 7 alpha-hydroxylated C27-steroids being as substrates in the liver [10]. Similar to plant sterols, DC is also poorly absorbed, having an absorption rate less than 3.3% [11]. This arouses our interest to study whether DC would possess a plasma TC - lowering activity like plant sterols.

The present study was to (i) study plasma TC - lowering activity of DC compared with that of β-sitosterol (SI), the major plant sterol, in hypercholesterolemia hamsters; and (ii) examine the effects of DC on the gene expression of sterol transporters, proteins, enzymes, and receptors involved in cholesterol absorption and metabolism. These include intestinal Niemann-Pick C1 like 1 protein (NPC1L1), acyl-CoA: cholesterol acyltransferase 2 (ACAT2), microsomal triacylglycerol transport protein (MTP) and ATP-binding cassette transporters sub-family G member 5 and 8 (ABCG5/8), as well as liver sterol regulatory element-binding protein 2 (SREBP-2), 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), low-density lipoprotein receptor (LDLR), Liver × receptor alpha (LXRα), and cholesterol-7α-hydroxylase (CYP7A1).

2. Methods and materials

2.1. Diets

Six diets were prepared (Table 1). The non-cholesterol diet (NCD) was prepared by mixing the following ingredients: 508 g corn starch, 242 g casein, 119 g sucrose, 50 g lard, 40 g mineral mix, 20 g vitamin mix, 1 g DL-methionine. The high cholesterol control diet (HCD) was prepared by adding 0.2% cholesterol (w/w) into NCD. The other four experimental diets were prepared by adding 0.2% DC (DA), 0.3% DC (DB), 0.2% SI (SA) and 0.3% SI (SB) into the HCD diet, respectively.

2.2. Hamsters

Forty-five male Golden Syrian hamsters (3 months, body weights = 100–120 g) were randomly divided into six groups

Table 1
Diet composition of non-cholesterol diet (NCD), high-cholesterol diet (HCD), and four experimental diets supplemented with 0.2% dihydrocholesterol (DA), 0.3% dihydrocholesterol (DB), 0.2% β-sitosterol (SA) and 0.3% β-sitosterol (SB), respectively.

Ingredients (g)	NCD	HCD	DA	DB	SA	SB
Corn starch	508	508	508	508	508	508
Casein	242	242	242	242	242	242
Sucrose	119	119	119	119	119	119
Lard	50	50	50	50	50	50
Mineral mixture AIN-76	40	40	40	40	40	40
Vitamin mixture AIN-76A	20	20	20	20	20	20
Gelatin	20	20	20	20	20	20
DL-methionine	1	1	1	1	1	1
Cholesterol	0	2	2	2	2	2
Dihydrocholesterol	0	0	2	3	0	0
β-Sitosterol	0	0	0	0	2	3

(n = 7 for NCD, HCD and DB, n = 8 for DA, SA, and SB) and fed one of the six diets for 6 weeks. All hamsters with one per cage were housed in wire-bottomed cages at 23 °C in an animal room with 12 h light–dark cycle. Diets and water were given *ad libitum*. All hamsters were weighed and their total fecal outputs per cage were collected weekly. After overnight fasting, 1 ml blood sample was obtained from the retro-orbital sinus and collected into a heparinized capillary tube under inhalational anesthesia of isoflurane (100%) at the beginning of week 1 and the end of week 6. Following the last blood sampling, all hamsters were sacrificed by carbon dioxide suffocation. The liver, heart, kidney, epididymal and perirenal adipose tissues were collected, washed in phosphate-buffered saline (PBS), and weighed. The first 5 cm of duodenum was discarded, and the next 30 cm of the small intestine was kept. All tissues were flash frozen in liquid nitrogen and stored at −80 °C until analysis. Thoracic aorta was collected, and stored in PBS after connective tissues were cleaned off. The entire experimental procedure was approved by the Animal Experimental Ethical Committee, the Chinese University of Hong Kong (Ref No: 13/006/mis).

2.3. Analysis of plasma lipoproteins

Plasma TC and total triacylglycerols (TG) were quantified using their respective commercial enzymatic kits (Infinity, Waltham, MA, USA and Stanbio Laboratories, Boerne, TX, USA.). To quantify plasma high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein and vey low-density lipoprotein cholesterol were firstly precipitated using a commercial kit containing phosphotungstic acid and magnesium chloride (Stanbio, Boerne, TX, USA). Following the centrifugation, HDL-C in the supernatant was measured as described for plasma TC. Non-HDL cholesterol (non-HDL-C) was calculated by reducing HDL-C from TC. It was found that DC in plasma could react with the enzymatic reagent with 1 mg being equivalent to that 0.137 mg of plasma cholesterol. Thus, GC analysis was performed to quantify % cholesterol (C) and % DC in plasma. Final plasma cholesterol reading was adjusted according to the following two equations: Factor (F) = % C ÷ (% C + 0.137 × % DC); Adjusted TC = Original TC × F.

2.4. Measurement of atherosclerotic plaque

The percentage area of atherosclerotic plaque in aorta was determined as previously described [12]. In brief, the thoracic aorta was cut opened vertically. The aortas were stained with saturated oil red (Sigma–Aldrich, St. Louis, MO, USA) in isopropanol before scanning (Epson 1220 Perfection, Epson Co., Tokyo, Japan). The area of atherosclerotic plaque was measured with the aid of computer