The Hypocholesterolemic and Antiatherogenic Effects of Cholazol H, a Chemically Functionalized Insoluble Fiber With Bile Acid Sequestrant Properties in Hamsters

Thomas A. Wilson, Christine Romano, Jinsheng Liang, and Robert J. Nicolosi

Cholazol H (Alpha-Beta Technology, Worcester, MA), a chemically functionalized, insoluble dietary fiber with bile acid sequestrant properties, was studied in 30 male F_1 B Golden Syrian hamsters for its effect on plasma lipid concentrations and early atherogenesis in experiment 1. In experiment 2, 30 male Golden Syrian hamsters were studied for the effects on plasma lipids and fecal excretion of bile acids. In experiment 1, three groups of 10 hamsters each were fed a chow-based hypercholesterolemic diet supplemented with 5% coconut oil and 0.1% cholesterol for 6 weeks. After 6 weeks, hamsters were continued on the diet with either 0% drug (hypercholesterolemic diet [HCD]), 0.5% cholestyramine (CSTY), or 0.5% Cholazol H for 8 weeks. Fasting plasma lipids were measured at weeks 6, 10, and 14, and early atherosclerosis (fatty streak formation) was measured at week 14. Relative to HCD, CSTY and Cholazol H significantly lowered plasma total cholesterol (TC) (−37%, $P < .03$, and −30%, $P < .04$, respectively) and plasma very-low and low-density lipoprotein-cholesterol (nonHDL-C) (−45%, $P < .02$, and −36%, $P < .03$, respectively) with no significant effects on plasma HDL-C or triglycerides (TG). Despite similar reductions in nonHDL-C, only Cholazol H significantly prevented early atherosclerosis (−38%, $P < .02$) relative to HCD. In experiment 2, three groups of 10 hamsters each were fed a chow-based hypercholesterolemic diet supplemented with 10% coconut oil and 0.05% cholesterol and either 0% drug HCD, 0.5% CSTY, or 0.5% Cholazol H for 4 weeks. Fasting plasma lipids were measured at weeks 2 and 4, and fecal bile acids were measured at week 4. Both Cholazol H and CSTY were equally effective in significantly lowering plasma TC (−16%, $P < .003$, and −13%, $P < .01$, respectively) and nonHDL-C (−22%, $P < .004$, and −18%, $P < .02$, respectively), with no significant effect on HDL-C and TG relative to HCD. Cholazol H and CSTY produced a significantly greater concentration of fecal total bile acids (39%, $P < .001$, and 28%, $P < .002$, respectively) relative to HCD. Also, there was a 48% ($P < .002$) and 65% ($P < .001$) greater fecal concentration of cholic acid (CA) for Cholazol H–treated hamsters compared with HCD- and CSTY-treated hamsters, respectively. Cholazol H also significantly increased fecal concentration of deoxycholic acid (DCA; 56%, $P < .02$) compared with HCD. In summary, Cholazol H is as effective as CSTY for prevention of diet-induced hypercholesterolemia and early atherosclerosis in hamsters.

ELEVATED BLOOD CHOLESTEROL is a major risk factor in the development of atherosclerosis and coronary heart disease, the leading cause of mortality in the United States. Various therapeutic approaches have been developed to reduce plasma cholesterol concentrations. In addition to dietary management, two major pharmacological drug categories are available, ie, systemic (absorbable) and nonsystemic (nonabsorbable) drugs, which rely on different mechanisms of action. Systemic drugs may be based on, among other mechanisms, the antagonism of hepatic cholesterol biosynthesis (hepatic hydroxymethyl glutaryl coenzyme A [HMG-CoA] reductase inhibitors, such as pravastatin and simvastatin), or on the activation of lipoprotein catabolism.[1,2] Nonsystemic drugs generally act at the intestinal level by affecting the absorption/excretion of neutral and acidic sterols. The hepatic synthesis of bile acids (ie, acidic sterols) from cholesterol is the major route of excretion and regulation of cholesterol homeostasis. Two nonsystemic agents, cholestyramine (CSTY) and colestipol, which are bile acid sequestrants (BAS), are polymeric drugs that are commonly used for the treatment of hypercholesterolemia when dietary management alone fails to control blood cholesterol concentrations. These BAS are nonabsorbable anion-exchange resins that are not altered by digestive enzymes. These drugs form a complex with bile acids in the intestine, which are then excreted in the feces. As a result of bile acids being removed from enterohepatic cycling, more hepatic cholesterol is converted to bile acids, resulting in an increase in hepatic low-density lipoprotein LDL receptor activity and lowering of plasma cholesterol concentrations. Of the two agents, CSTY is the more widely prescribed for patients with hypercholesterolemia.

The disadvantages of the synthetic nonsystemic cholesterol-lowering agents are their unfavorable amine odor and palatability characteristics (grittiness, sand-like mouth feel), and the large doses of these compounds that are required to be ingested for maximal therapeutic benefit, ie, 8 to 24 g/d and 15 g/d for CSTY[3] and colestipol,[4] respectively. In addition, the solids of such compounds do not readily disperse in water, tend to clump, and rapidly settle in aqueous solutions. Although CSTY can lower plasma cholesterol concentrations, these disadvantages are associated with patient noncompliance.

There has been considerable interest in using nonsystemic, carbohydrate-derived materials for the treatment of hypercholesterolemia. Numerous reports have described the use of, among others, various natural fibers,[5-7] psyllium,[8,9] cyclodextrins,[10] guar gum,[11] and pectin,[12] as well as chemically modified materials, such as hydroxypropylmethylcellulose[13] and methylcellulose.[14] The cholesterol-lowering features of these polysaccharides, or soluble dietary fibers, have been ascribed to the role of viscosity builders in the intestines, which reduce absorption

From The Center for Chronic Disease Control, Department of Health and Clinical Science, University of Massachusetts Lowell, Lowell, MA. and Alpha-Beta Technology, Worcester, MA.

Submitted September 8, 1997; accepted February 4, 1998.

Supported by Alpha-Beta Technology (R.J.N.), and National Institutes for Health Grant No. HL55044-01 (R.J.N.).

Address reprint requests to Robert J. Nicolosi, PhD, The Center for Chronic Disease Control, Department of Health and Clinical Science, Room 305, Weed Hall, University of Massachusetts Lowell, Lowell, MA 01854.

0026-0495/98/4708-0012$03.00/0

of all food ingredients, including cholesterol and bile acids. The disadvantages of the natural materials are their low level effectiveness in lowering plasma cholesterol concentrations, absorption of substantial amounts of water, and gastrointestinal pain, nausea, bloating, flatulence, and laxation associated with the need to consume large amounts, ie, up to 100 g/d.

Previous studies examined the cholesterol-lowering potential of whole glucan particles (WGP), spherical β-glucan particles derived from baker's yeast.[15] These particles are approximately 3 to 5 μm in diameter and impart a smooth mouth feel similar to some other fat mimetics, a feature that is believed to facilitate higher patience compliance.[15] WGP have been shown to reduce plasma cholesterol concentrations in both hamsters and humans.[15] Since WGP do not bind bile acids or cholesterol significantly in vitro, the mechanism does not appear to involve dietary lipid sequestration (unpublished observations, October 1994). Unlike some other polysaccharides, WGP do not form gels in water and are not digested.[15] Thus, it was postulated that their efficacy could be improved by transforming the hydrophilic carbohydrate into a matrix with higher affinity for bile acids.

Cholazol H (Alpha-Beta Technology, Worcester, MA) is a chemically functionalized, insoluble dietary fiber that combines the cholesterol-lowering properties of dietary fiber with the BAS properties of a hydrophobic catonized resin. Specifically, Cholazol H was formed by functionalizing WGP with alkylamine residues. The additional quaternization of the nitrogen residues would facilitate ionic interactions with bile anions, thus affording a hydrophobic anion-exchange resin. The selected modifications involved oxidation activation with sodium periodate of the WGP substrate, and subsequent incorporation, via reductive amination, and quaternization (permethylation) of an alkyldiamine ligand (1, 12-dodecanediamine).[16] Various compounds were prepared and evaluated in vitro for their chloride ion-exchange and bile acid–binding capacity. Based on these results, Cholazol H (a methylated dodecanediamine ligand) was found to have both high taurocholate (TCA)-binding capacities and TCA-binding:chloride ion-exchange ratios.[16] Therefore the specific aims of this study were as follows: (1) to compare Cholazol H with CSTY in preventing diet-induced hypercholesterolemia and early atherogenesis in hamsters, and (2) to examine mechanism(s) of action of Cholazol H.

MATERIALS AND METHODS

Animals and Diets

In experiment 1, 30 male F_1 B Golden Syrian hamsters (BioBreeders, Fitchburg, MA), approximately 10 weeks of age, were divided into three groups of 10 animals per group, housed in individual hanging cages, and fed a hypercholesterolemic chow-based diet (Purina Chow 5001; Ralston Purina, St Louis, MO) supplemented with 5% coconut oil and 0.1% cholesterol for 6 weeks. After 6 weeks, the hamsters were then additionally fed either 0% drug (hypercholesterolemic diet [HCD]), 0.5% CSTY (wt/wt) (Sigma Chemical, St Louis, MO), or 0.5% Cholazol H (wt/wt) for 8 weeks. In experiment 2, 30 male F_1 B Golden Syrian hamsters, approximately 10 weeks of age, were divided into three groups of 10 animals per group, housed in individual hanging cages, and fed a hypercholesterolemic chow-based diet supplemented with 10% coconut oil and 0.05% cholesterol plus 0% HCD, 0.5% CSTY (wt/wt), or 0.5% Cholazol H (wt/wt) for 4 weeks. A chow-based, rather than a semipurified diet, was used, because published data from our laboratory[17] and those from another[18] have demonstrated that the chow-based diet produced a lipoprotein profile (predominantly non–high-density lipoprotein-cholesterol [nonHDL-C]) more similar to humans. Also, the use of a higher cholesterol diet in experiment 1 was to produce atherogenesis in the hamsters within the 8-week treatment period. However, to produce the same degree of hypercholesterolemia, a higher amount of coconut oil was necessary in experiment 2. All animals had free access to food and water. Animals were maintained in accordance with the guidelines of the Committee on Animals of the University of Massachusetts at Lowell Research Foundation and the guidelines prepared by the Committee on Care in Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication no. 85-23, revised 1985). All animals were maintained in American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities, in an environmentally controlled atmosphere (20°C) on a 12-/12-hour light/dark cycle.

Lipid Measurements

In experiment 1, fasting blood samples were collected at weeks 6, 10, and 14 from the retroorbital sinus into heparinized capillary tubes and plasma was harvested by low-speed centrifugation at 1,500 rpm at 4°C. Plasma was frozen at −80°C until analyzed for plasma total cholesterol (TC), HDL-C, very–low-density lipoprotein-cholesterol (VLDL-C) and LDL-C (nonHDL-C), and triglycerides (TG). In experiment 2, fasting blood samples were collected at weeks 2 and 4 via the same procedure, with the same plasma lipid measurements performed. Plasma cholesterol[19] and TG[20] levels were measured enzymatically and, after the apolipoprotein (apo) B-containing lipoproteins VLDL and LDL were precipitated with phosphotungstate reagent,[21] the supernatant was assayed for HDL-C (Sigma). Plasma nonHDL-C was calculated from the difference between TC and HDL-C. Plasma lipid determinations are standardized by participation in the Center for Disease Control–National Heart, Lung, and Blood Institute Standardization Program.

Quantification of Aortic Fatty Streak Area

In experiment 1, at the end of the treatment period, hamsters were anesthetized with an intraperitoneal injection of sodium pentobarbital and aortic tissue was obtained for fatty streak analysis as previously described.[22,23] A perfusion needle was inserted through the left ventricle and 10% buffered formalin solution was perfused through the animal's cardiovascular system under physiological pressure. The right atrium was punctured to provide an outlet for the fixative. After 2 minutes, the flow of fixative was slowed, and fixation continued for an additional 25 minutes. The heart and thoracic aorta were removed and fixation completed by pinning the tissue down in a metal pan containing dental wax overnight in its natural shape in 10% formalin. Specimens were stored in vials containing phosphate-buffered saline at 4°C for subsequent analysis of fatty streak area. To measure the extent of the fatty streak formation in the aortic arch, a piece of aortic tissue extending from as close to the heart as possible to the branch of the left subclavian artery was used. The tissue was cleaned, rinsed quickly with isopropanol, and placed in a vial containing 3% Oil Red O (ORO) stain (0-0625, Sigma Chemical) in 60% isopropanol for 15 minutes. Subsequently, the ORO-stained tissue was carefully and gently removed from the outside surface of the arch. The arch was dissected vertically in two, then cut open longitudinally along the inferior border, and mounted on a glass slide, endothelial side up. Specimens were covered with Apathy's mounting media and cover slips, and analyzed under 145x magnification. A computerized image analysis system (Image Technology, Cresskill, NJ), attached to a compound light microscope, was used to measure the total ORO-stained area of each aortic arch, and size of the arch was measured (SigmaScan; Jandel Scientific, San Rafeal, CA) to calculate the fatty streak area (square microns) per aortic arch area (square millimeters).

Fecal Bile Acid Measurements

In experiment 2, feces were collected over the final 3 days for fecal bile acid analysis. Dry feces (200 mg) were extracted with 4 mL of methanol/water (80:20) for 1 hour at 100°C in a 5-mL Reacti-vial fitted with a mini-nert cap (Pierce, Rockford, IL). Samples were then allowed to come to room temperature and centrifuged at 500 × *g* at room temperature for 10 minutes. The supernatant was removed from the fecal pellet and transferred to an 8-mL borosilicate vial. A second extraction of the same fecal pellet was performed using 4 mL of methanol/chloroform (50:50) and a third extraction using 4 mL of 1-mol/L ammonium carbonate/methanol (20:80) using the same conditions as the first extraction. The three supernatants were pooled and evaporated to dryness at 100°C under N_2.

Four milliliters of 0.1N NaOH/ethanol (10:90 by volume) were added to each sample over layered with N_2, capped, and heated at 100°C for 30 minutes. The samples were allowed to cool to room temperature followed by the removal of the solvent, which was transferred to 16- × 150-mm borosilicate test tubes. Five milliliters of water and 3 mL of hexane were added to the solvent followed by vortexing and centrifugation at 500 × *g* for 2 minutes. The top hexane layer was removed and placed in vials. The hexane extraction was repeated two more times.

The aqueous layer was brought up to 10 mL with the addition of 0.1NNaOH. One milliliter was removed for quantitative enzymatic determination of total bile acids. To the remaining volume, 1 mL of 23 nor-5-β-cholanic acid (80 μg/mL) was added. The sample was directly loaded onto a prewashed Sep-Pak C18 cartridge (Waters Chromatography, Milford, MA). Prewashing conditions included 5 mL of methanol, 5 mL of 50% methanol, and 5 mL of water. After loading the sample, the cartridge was washed with 20 mL of water followed by 4 mL of 2% methanol. Bile acids were eluted with 4 mL of 100% methanol and collected. The methanol eluate was brought up to 10 mL in a volumetric flask with the addition of methanol and exactly 4 mL was removed and evaporated to dryness at 100°C under N_2. Two milliliters of 1% HCl in methanol was added to each sample, then overlayered with N_2 and heated at 80°C for 1 hour. The samples were evaporated to dryness at 100°C under N_2, followed by addition of 100 μL of Tri-Sil reagent (Pierce, Rockford, IL) and heated at 85°C for 20 mintues. Evaporated samples were reconstituted with 100 μL of acetonitrile. One microliter was then analyzed by capillary gas chromatography (GC).

GC Analyses

Bile acids were analyzed using a Shimadzu GC-14A gas chromatograph with a flame ionization detector (Kyoto, Japan) and a 50-m × 0.2-mm HP-1 capillary column (Hewlett Packard, Andover, MA). The injector and detector temperatures were set at 300°C. The initial column temperature was 220°C and was increased to 300°C at a rate of 2°C/min. The final temperature was held for 10 minutes. Column flow rate was 1.5 mL/min. Peak areas were quantitated using a Shimadzu CR501 integrator.

Determination of Fecal Total Bile Acids by Enzymatic Assay

Fecal total bile acids were measured enzymatically according to the protocol provided with the Sigma Bile Acids Kit #450-A.

Statistical Analyses

A one-way ANOVA was used to examine the effect of treatment on the different variables using SigmaStat (Jandel Scientific). Differences between group means were assessed by the Student-Newman-Keuls test. All values are expressed as the mean ± SEM and statistical significance was set at $P < .05$.[24]

RESULTS

Experiment 1

The initial body weights for the hamsters on the HCD, Cholazol H, and CSTY treatments were 105 ± 2.3, 103 ± 4.0, and 105 ± 2.4 g, respectively, and the final body weights were 127 + 2.8, 126 + 3.9, and 126 ± 1.4 g, respectively. Although the hamsters gained a significant amount of body weight during the treatment period, this weight gain was similar in the three groups.

The results of plasma lipid concentrations and aortic fatty streak area in the hamsters are shown in Table 1. After feeding the hypercholesterolemic diet to the hamsters for the first 6 weeks, plasma lipids were not significantly different from each other (data not shown).

Since plasma lipid concentrations at 10 and 14 weeks within each treatment were not significantly different from each, the two measurements were averaged (Table 1). Both CSTY and Cholazol H significantly lowered plasma TC (−37%, $P < .03$, and −30%, $P < .04$, respectively) and nonHDL-C (−45%, $P < .02$, and −36%, $P < .03$, respectively) relative to HCD. Both CSTY and Cholazol H had lower plasma TG (−58% and −54%, respectively) and HDL-C (−6% and −4%, respectively) levels relative to HCD, but these reductions were not statistically significant. Neither CSTY or Cholazol H was significantly different from each other for any plasma lipid measurement (Table 1).

Only Cholazol H significantly reduced aortic fatty streak area (−38%, $P < .02$) relative to HCD. However, CSTY did reduce aortic fatty streak area by −25% relative to HCD (Table 1). Also, aortic fatty streak area was not significantly different between CSTY and Cholazol H.

Experiment 2

The initial body weights for the HCD, Cholazol H, and CSTY hamsters were 111 ± 3.1, 113 ± 2.1, and 113 ± 2.2 gr, respectively, and the final body weights were 122 ± 3.5, 120 ±

Table 1. Plasma Lipid Concentrations and Aortic Fatty Streak Area of Hamsters in Experiment 1 (average of weeks 10 and 14 measurements)

Group	TC (mmol/L)	nonHDL-C (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)	AFSA ($\mu m^2 \times 10^3/mm^2$)
HCD	11.53 ± 1.56†	9.14 ± 1.48†	2.39 ± 0.18	7.41 ± 1.91	64.9 ± 7.8†
CSTY	7.28 ± 0.74*	5.03 ± 0.66*	2.25 ± 0.12	3.09 ± 0.76	48.4 ± 5.4
Cholazol H	8.11 ± 0.72†	5.81 ± 0.69†	2.30 ± 0.11	3.43 ± 0.78	40.0 ± 3.1†

NOTE. Values are means ± SEM; n = 10.
Abbreviation: AFSA, aortic fatty streak area.
*$P < .05$, HCD *v* CSTY.
†$P < .05$, HCD *v* Cholazol H.

4.3, and 122 ± 3.0 g, respectively. Although the hamsters gained a significant amount of body weight during the treatment period, the weight gain was similar in the three groups.

The results of plasma lipid concentrations in the hamsters after 4 weeks of dietary treatment are shown in Table 2. Since the 2- and 4-week plasma lipid concentrations were not significantly different within drug treatments, the two measurements were averaged. After 4 weeks, Cholazol H and CSTY had significantly lower plasma TC and nonHDL-C concentrations relative to HCD (−16%, $P < .003$, and −13%, $P < .01$, and −23%, $P < .004$, and −18%, $P < .02$, respectively). Plasma HDL-C and TG concentrations were not significantly different from each other for any group. Both Cholazol H and CSTY hamsters had lower HDL-C concentrations relative to HCD hamsters (−6%).

The total fecal output for the HCD, Cholazol H, and CSTY hamsters were not significantly different from each other (3.35 ± 0.31, 3.00 ± 0.33, and 2.86 ± 0.18 g over 3 days, respectively). The results of fecal bile acid analysis for the groups are shown in Table 3. Relative to HCD, Cholazol H and CSTY treatments significantly increased fecal concentration of total bile acids by 64% ($P < .001$) and 39% ($P < .002$), respectively. Relative to HCD and CSTY, Cholazol H significantly increased fecal concentration of cholic acid (CA) by 94% ($P < .002$) and 182% ($P < .001$), respectively. CSTY decreased fecal concentration of CA by −31%, but not significantly compared with HCD. Cholazol H had significantly higher fecal concentration of chenodeoxycholic acid (CDCA) (0.21 ± 0.02 mg/g dry feces) compared with both CSTY (0.09 ± 0.09 mg/g dry feces; $P < .0002$) and HCD (0.00 ± 0.00 mg/g dry feces; $P < .0001$). Relative to HCD, Cholazol H significantly increased the fecal concentration of deoxycholic acid (DCA) (126%, $P < .02$). Although not significant, CSTY increased the fecal concentration of DCA by 86%, relative to HCD. Relative to HCD, Cholazol H produced an equal concentration of lithocholic acid (LCA) (−1%), whereas CSTY produced a 19% increase in LCA concentration; however, these differences were not significant (Table 3).

DISCUSSION

In experiment 2, hamsters were placed on the different dietary treatments after a 1-week acclimation period where they were fed chow, whereas in experiment 1, hamsters were fed a hypercholesterolemic diet for 6 weeks before placed on the different dietary treatments.

In experiment 1 of the present hamster study, the insoluble fiber-based BAS Cholazol H versus the same dose of CSTY reduced plasma TC (−30% *v* −37%), nonHDL-C (−36% *v* −45%), HDL-C (−4% *v* −6%), and TG (−54% *v* −58%) levels relative to HCD after 8 weeks on the diets. Also in experiment 1, Cholazol H significantly reduced aortic fatty streak area by −38% relative to HCD, whereas CSTY treatment did not significantly reduce aortic fatty streak area (−25%) relative to HCD.

Table 2. Plasma Lipid Concentrations of Hamsters in Experiment 2 (average of weeks 2 and 4 measurements)

Group	TC (mmol/L)	NonHDL-C (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)
HCD	4.97 ± 0.17*†	2.75 ± 0.15*†	2.21 ± 0.04	2.95 ± 0.14
CSTY	4.32 ± 0.15*	2.25 ± 0.12*	2.07 ± 0.06	3.62 ± 0.35
Cholazol H	4.20 ± 0.15†	2.14 ± 0.11†	2.07 ± 0.07	3.05 ± 0.18

NOTE. Values are means ± SEM; n = 10.
*$P < .05$, HCD *v* CSTY.
†$P < .05$, HCD *v* Cholazol H.

Table 3. Total Fecal Bile Acid Concentrations of Hamsters in Experiment 2 (mg/g of dry feces)

Bile Acid	HCD	CSTY	Cholazol H
LCA	1.06 ± 0.12	1.26 ± 0.11	1.05 ± 0.07
DCA	0.69 ± 0.09†	1.28 ± 0.21	1.56 ± 0.19†
CDCA	ND†	0.09 ± 0.09‡	0.21 ± 0.02†‡
CA	0.16 ± 0.05†	0.11 ± 0.02‡	0.31 ± 0.05†‡
Total§	1.91 ± 0.21†	2.66 ± 0.27*	3.13 ± 0.26*†

NOTE. Values are means ± SEM; n = 10.
Abbreviation: ND, none detected.
*$P < .05$, HCD *v* CSTY.
†$P < .05$, HCD *v* Cholazol H.
‡$P < .05$, CSTY *v* Cholazol H.
§Total fecal bile acids were measured by enzyme assay, whereas individual values were calculated from % distribution from GC analysis.

In experiment 2, Cholazol H exhibited equal efficacy in preventing the development of hypercholesterolemia to CSTY in the hypercholesterolemic hamster model. Cholazol H versus an equal dose of CSTY had similar plasma TC (−16% *v* −13%), LDL-C (−22% *v* −18%), HDL-C (−6% *v* −6%), and TG (+4% *v* +16%) concentrations relative to HCD after 4 weeks of treatment. The effect of BAS on lowering plasma TC, HDL-C, and LDL-C concentrations has been previously reported.[25-27] The proposed mechanism responsible for the LDL-C–lowering effect of CSTY feeding in humans is an increase clearance of the lipoprotein by the LDL receptor–mediated pathway.[27] Also, it has been suggested that a decreased rate of VLDL-C production is another possible mechanism for lowering LDL-C in hamsters.[27]

Alteration in the levels of HDL-C in CSTY-treated hamsters has been previously reported.[27,29,30] In species in which the HDL-C contains high apo E concentrations, there may be an increase in uptake of HDL-C by the LDL receptor.[29] Hamster HDL-C contains apo E and may thus be removed via LDL receptors.[27] It should be noted that BAS does not decrease HDL-C in humans and may even cause a small increase.[30] The difference between hamsters and humans in the response to BAS treatment may be related to the higher plasma cholesterol concentrations and higher proportion of TC in LDL-C in humans. Thus, when demand for cholesterol increases due to BAS treatment in humans, the LDL-C fraction alone can compensate for the demand, whereas in hamsters LDL-C is in insufficient quantities and HDL-C is reduced. Also, the LDL receptors in hamsters treated with BAS will be upregulated and may be the reason for the reduction in plasma HDL-C concentrations.

Cholazol H increased fecal total bile acid concentration versus CSTY treatment (64% *v* 39%) in hamsters relative to HCD. Many previous studies have also reported increases in

fecal total bile acid concentration in hamsters treated with CSTY.[3,25,26,31] However, a unique finding in the present study was the effect of Cholazol H on the individual fecal bile acids. While CSTY treatment had no significant effect on fecal primary bile acids CA (−31%) and CDCA concentration relative to HCD, Cholazol H significantly increased fecal CA (94%) and CDCA concentrations. Also, Cholazol H hamsters had significantly higher CA concentrations relative to CSTY hamsters (182%). Cholazol H decreased and CSTY increased the fecal concentration of LCA (−1% and +19%, respectively) relative to HCD. Cholazol H significantly increased the concentration of DCA (126%) relative to HCD, while CSTY treatment had no significant effect on DCA concentration relative to HCD.

Both in vitro[32] and in vivo[33] evidence suggest that CSTY preferentially binds and prevents reabsorption of CDCA, which leads to an increased conversion and fecal concentration of LCA. Cholazol H appears to have a slightly greater binding effect on CDCA and CA than did CSTY in the present hamster study, and as was observed in an in vitro study.[16] However, due to the binding of CDCA to Cholazol H, there was a slight decrease in fecal concentration of LCA, which is indicative of less free CDCA available for bacterial conversion into LCA.

In the present study, Cholazol H was shown to be as effective as CSTY for the prevention of diet-induced hypercholesterolemia and early atherosclerosis in hamsters. The results demonstrate the feasibility of developing palatable carbohydrate-based functionalized nonsystemic compounds as viable cholesterol-lowering drug candidates.

ACKNOWLEDGMENT

The authors would like to thank Donato Vespa, Lorraine Misner, and Lam Tram for their technical support.

REFERENCES

1. Sirtori C, Manzoni C, Lovati M: Mechanisms of lipid-lowering agents. Cardiology 78:226-235, 1991

2. Stedronsky R: Interaction of bile acids and cholesterol with non-systemic agents having hypocholesterolemic properties. Biochim Biophys Acta 1210:255-287, 1994

3. Suckling KE, Benson GM, Bond B, et al: Cholesterol lowering and bile acid excretion in the hamster with cholestyramine treatment. Atherosclerosis 89:183-190, 1991

4. Day CE: Comparison of hypocholesterolemic activities of the bile acid sequestrants cholestyramine and colestipol hydrochloride in cholesterol-fed sea quail. Artery 17:281-288, 1990

5. Anderson JW, Jones AE, Riddell-Mason S: Ten different dietary fibers have significantly different effects on serum and liver lipids of cholesterol-fed rats. J Nutr 124:78-83, 1994

6. Overton PD, Furlonger N, Beety JM, et al: The effects of dietary sugar-beet fibre and guar gum on lipid metabolism in Wistar rats. Br J Nutr 72:385-395, 1994

7. Tinker LF, Davis PA, Schneeman BO: Prune fiber or pectin compared with cellulose lowers plasma and liver lipids in rats with diet-induced hyperlipidemia. J Nutr 124:31-40, 1994

8. Daggy BP, O'Connell NC, Jerdack GR, et al: Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: Influence on fecal sterol and bile acid profiles. J Lipid Res 38:491-502, 1997

9. Sprecher DL, Harris BV, Goldberg AC, et al: Efficacy of psyllium in reducing serum cholesterol levels in the hypercholesterolemic patients on high- or low-fat diets. Ann Intern Med 119:545-554, 1993

10. Riottot M, Olivier P, Huet A, et al: Hypolipidemic effects of β-cyclodextrin in the hamster and in the genetically hypercholesterolemic Rico rat. Lipids 28:181-188, 1993

11. Todd PA, Benfield P, Goa KL: Guar gum. A review of its pharmacological properties, and use as a dietary adjunct in hypercholesterolemia. Drugs 39:917-928, 1990

12. Lewinska D, Rosinski S, Piatkiewicz W: A new pectin-based material for selective LDL-cholesterol removal. Artif Organs 18:217-222, 1994

13. Dressman B, Adair C, Barnett JL: High-molecular-weight hydroxypropylmethyl cellulose. Arch Intern Med 153:1345-1353, 1993

14. Topping DL, Oakenfull D, Trimble RP, et al: A viscous fibre (methylcellulose) lowers blood glucose and plasma triacylglycerols and increases liver glycogen independently of volatile fatty acid production in rats. Br J Nutr 59:21-30, 1988

15. Ostroff G: U.S. Patent No. 481066, 4992540, 5037972, 5082936, 5028703, 4962094 and U.S. Patent Appl. Serial No. 07/675913, Glucan dietary additives, 1991

16. Stevenson T, Liang J, Ostroff G, et al: U.S. Paten Appl. Serial No. 08/146225, A derivatized polysaccharide bile acid sequestrant for reducing cholesterol, 1993

17. Terpstra AHM, Holmes JC, Nicolosi RJ: The hypocholesterolemic effect of dietary soybean protein vs casein in hamsters fed cholesterol-free or cholesterol-enriched semipurified diets. J Nutr 121:944-947, 1991

18. Krause BR, Bousley RF, Kieft KA, et al: Effect of the ACAT inhibitor Cl-976 on plasma cholesterol concentrations and distribution in hamsters fed zero- and no-cholesterol diets. Clin Biochem 25:371-377, 1992

19. Allain CC, Poon LS, Chen CSG, et al: Enzymatic determination of total serum cholesterol. Clin Chem 20:470-475, 1974

20. Buccolo G, David H: Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 36:476-482, 1973

21. Weingand KW, Daggy BP: Quantification of high-density lipoprotein cholesterol in plasma from hamsters by differential precipitation. Clin Chem 36:575, 1990

22. Foxall TL, Shwaery GT, Stucchi AF, et al: Dose-response effects of doxazosin on plasma lipids, lipoprotein cholesterol, and aortic fatty streak formation in hypercholesterolemic hamsters. Am J Pathol 140:1357-1363, 1992

23. Kowala MC, Nunnari JJ, Durham SK, et al: Doxazosin and cholestyramine similarly decrease fatty streak formation in the aortic arch of hyperlipidemic hamsters. Atherosclerosis 91:35-49, 1991

24. Snedecor GW, Cochran WG: Statistical Methods. Ames, IA, Iowa State University Press, 1980

25. Benson GM, Alston DR, Bond BC, et al: SK&F 97246—A a more potent bile acid sequestrant and hypocholesterolemic agent than cholestyramine in the hamster. Atherosclerosis 101:51-60, 1993

26. Trautwein EA, Siddiqui A, Hayes KC: Modeling plasma lipoprotein-bile-lipid relationships: Differential impact of psyllium and cholestyramine in hamsters fed a lithogenic diet. Metabolism 42:1531-1540, 1993

27. Groot PHE, Pearce NJ, Suckling KE, et al: Effects of cholestyramine on lipoprotein levels and metabolism in Syrian hamsters. Biochim Biophys Acta 1123:76-84, 1991

28. Packard CJ, Shepherd J: The hepatobiliary axis and lipoprotein metabolism: Effects of bile acid sequestrants and ileal bypass surgery (review). J Lipid Res 23:1081-1098, 1982

29. Mahley RW, Hui DY, Innerarity TI, et al: Two independent

lipoprotein receptors on hepatic membranes of dog, swine, and man. Apo-B,E and Apo-E receptors. J Clin Invest 678:1197-1206, 1981

30. Hunninghake DB: Bile acid sequestrant therapy. J Drug Dev 3:205-211, 1990 (suppl 1)

31. Singhal AK, Finver-Sadowsky J, McSherry CK, et al: Effect of cholesterol and bile acids on the regulation of cholesterol metabolism in hamsters. Biochim Biophys Acta 752:214-222, 1983

32. Grundy SM, Ahrens EH Jr, Salen G: Interruption of the enterohepatic circulation of bile acids in man: Comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. J Lab Clin Med 78:94-121, 1971

33. Garbutt JT, Kenney TJ: Effect of cholestyramine on bile acid metabolism in the mouse as affected by cholestyramine. Proc Soc Exp Biol Med 122:881-884, 1966