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SC-435, an ileal apical sodium-codependent bile acid transporter inhibitor alters mRNA levels and enzyme activities of selected genes involved in hepatic cholesterol and lipoprotein metabolism in guinea pigs

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Abstract

We have demonstrated that SC-435, an apical sodium codependent bile acid transporter (ASBT) inhibitor, lowers plasma low-density lipoprotein cholesterol (LDL-C) concentrations in guinea pigs. The purpose of this study was to further examine the hypocholesterolemic effects of SC-435, by measuring the activity and RNA expression of regulatory enzymes of hepatic cholesterol and lipoprotein metabolism. In addition, the use of a combination (COMBO) therapy with simvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, was also tested. Male Hartley guinea pigs were randomly allocated to one of three diets ($n=10$ per group), for 12 weeks. The control diet contained no ASBT inhibitor or simvastatin. The monotherapy diet (ASBTi) contained 0.1% of SC-435. The COMBO therapy consisted of a lower dose of SC-435 (0.03%) and 0.05% simvastatin. Cholesterol ester transfer protein (CETP) and HMG-CoA reductase mRNA abundance were determined using RT-PCR techniques. Hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase (CYP7) activities were measured by radioisotopic methods. Compared to the control group, CETP activity was 34% and 56% lower with ASBTi and COMBO, respectively. Similarly, CETP mRNA expression was reduced by 36% and 73% in ASBTi and COMBO groups, respectively. Cholesterol 7α-hydroxylase and HMG-CoA reductase activities were increased ~2-fold with ASBTi and COMBO treatments, respectively. Likewise, HMG-CoA reductase mRNA expression was increased 33% with ASBTi treatment. These results suggest that both SC-435 monotherapy and combination therapy lower LDL cholesterol concentrations by altering both hepatic cholesterol homeostasis and the intravascular processing of lipoproteins in guinea pigs.

Keywords: ASBT inhibitor; CETP; Cholesterol 7 alpha hydroxylase; HMG-CoA reductase; Guinea pigs

1. Introduction

Significant reductions in plasma low-density lipoprotein cholesterol (LDL-C) concentrations have been related to modifications in the enterohepatic circulation of bile acids, which in turn can alter hepatic cholesterol and plasma lipoprotein metabolism [1]. Conversion of hepatic cholesterol to bile acids represents the major regulatory pathway by which the body eliminates excess cholesterol [2]. Hepatic cholesterol 7α-hydroxylase (CYP7) is the rate limiting enzyme in the classic bile acid biosynthesis pathway. Cholesterol 7α-hydroxylase expression is subject to negative feedback regulation by bile acids returning to the liver via enterohepatic circulation [3], thereby ensuring that bile acid synthesis is strictly controlled [4].

After synthesis in the liver, bile acids are secreted into the small intestine where they facilitate absorption of fat, fat soluble vitamins and cholesterol [5]. Bile acids are then reabsorbed and returned to the liver via portal circulation. Ileal bile acid transport is mediated by the apical sodium codependent bile acid transporter (ASBT) [6].

Decreased delivery of bile acids to the liver by ASBT inhibition could alleviate the feedback inhibition of bile acids and therefore increase CYP7 activity. Because cholesterol is the precursor to bile acid synthesis, changes in hepatic cholesterol synthesis may result from ASBT inhibition. Hepatic cholesterol synthesis can be evaluated by measuring 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in the liver.

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Animal studies have documented significant decreases in plasma LDL-C concentrations with ASBT inhibition [2,7,8]. In addition to reducing LDL-C concentrations, ASBT inhibition may elicit effects on the intravascular processing of lipoproteins. High-density lipoprotein (HDL) cholesteryl esters (CE) can be transported either directly to the liver [9,10] or to apoB-containing lipoproteins by cholesterol ester transfer protein (CETP) for hepatic uptake [11]. Cholesterol ester transfer protein may be viewed as either pro- or anti-atherogenic. The pro-atherogenic nature of CETP is related to its ability to transfer CE from anti-atherogenic HDL particles to VLDL and LDL particles, which then become cholesterol enriched and therefore more atherogenic [12]. On the other hand, CETP plays a crucial role in reverse cholesterol transport. Overall, the rate of CE transfer to VLDL and LDL fractions, as well as CETP activity, is increased in patients with atherogenic dyslipidemias [13].

SC-435, is a specific, nonabsorbable, inhibitor of ASBT function in the distal ileum [14,15]. Previous guinea pig studies in our laboratory have shown that LDL-C is significantly reduced by 40% and 70% with a monotherapy of 47.8 mg/kg per day SC-435 and a combination therapy (COMBO) of 13.7 mg/kg per day SC-435 and 21.4 mg/kg per day simvastatin, respectively [1]. Plasma triglyceride (TG) was 70% lower with the COMBO therapy ($P<.05$), while HDL cholesterol (HDL-C) was 74–87% higher with both treatments ($P<.05$). In addition, hepatic free cholesterol was reduced 60–80% with both treatments ($P<.01$). Finally, cholesterol content in the aortic arch was reduced by 25% and 42% in the SC-435 and COMBO groups, respectively [1]. These data suggest that the interruption in the enterohepatic circulation of bile acids by the SC-435 monotherapy and combination therapy plays a significant role in reducing cholesterol concentrations [9] and the progression of atherosclerosis in guinea pigs [1].

The current study was conducted to further explore the mechanisms by which the ASBT inhibitor monotherapy and combination therapy lower plasma cholesterol levels in the guinea pig. We hypothesized that the cholesterol lowering properties of SC-435 would be related to decreases in CETP activity and mRNA expression, while CYP7 and HMG-CoA reductase activities would be increased. We also hypothesized that the combination therapy would enhance the activity and expression of these proteins. For the purpose of this study, hepatic CYP7 and HMG-CoA reductase activities and CETP and HMG-CoA reductase mRNA expressions were analyzed from livers isolated from the guinea pigs whose plasma and hepatic lipids have previously been reported [1]. Furthermore, plasma CETP activity was determined from the same animals. Guinea pigs were used as the animal model for this study because of their similarities to humans in terms of hepatic cholesterol and lipoprotein metabolism [16] and their documented response to drug treatment [17].

2. Materials and methods

2.1. Materials

Diets were prepared and pelleted by Research Diets (New Brunswick, NJ). Cholesterol kits were purchased from Boehringer Mannheim (Indianapolis, IN). Free cholesterol kits were obtained from Wako (Osaka, Japan). 7α-Hydroxycholesterol and 7β-hydroxycholesterol standards were purchased from Steraloids (Newport, RI). ^{14}C Cholesterol was obtained from Perkin Elmer. [1-^{14}C] Cholesterol (1.8 GBq/mmol) and DL-HMG-CoA were obtained from Amersham (Clearbrook, IL). Aluminum and glass silica gel plates were purchased from EM Science (Gibbstown, NJ). Trizol reagent was purchased from Life Technologies (Carlsbad, CA). Oligonucleotide primers were obtained from Sigma Genosys (The Woodlands, TX). The One-Step RT-PCR kit was purchased from Qiagen (Valencia, CA). SC-435 and simvastatin were provided by Pharmacia (St. Louis, MO).

2.2. Diets

Isocaloric diets were designed to meet the nutritional requirements of the guinea pigs. All diets were equal in composition except for the amount of SC-435 and simvastatin. Diet I was a control diet with no SC-435 or simvastatin. Diet II was a monotherapy (ASBTi) containing 0.1 g/100 g SC-435, and Diet III was a combination therapy (COMBO) containing 0.03 g/100 g SC-435 and 0.05 g/100 g simvastatin. The amount of cholesterol in all diets was maintained at 0.25 g/100 g in order to raise plasma cholesterol concentrations to more readily detect SC-435 effects. Dietary cholesterol of 0.25% in this model corresponds to an absorbed amount equal to the daily cholesterol synthesis rates [18] in guinea pigs and is equivalent to 1800 mg/day for a human diet. The fat mix was rich in lauric and myristic acids, known to cause endogenous hypercholesterolemia in guinea pigs [19].

2.3. Animals

Male Hartley guinea pigs weighing between 250 and 300 g were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Animals, 10 guinea pigs per group, were randomly allocated to one of three treatments for 12 weeks: control, ASBTi or COMBO. Two guinea pigs were housed per cage, in a light cycle room (light from 7:00 a.m. to 7:00 p.m.), at 72°F. Diet and water were provided ad libitum. Each guinea pig was housed individually for 48 h after approximately 11.5 weeks of treatment. During this time, diets were weighed daily to determine the amount of food consumed. All animal experiments were conducted in accordance with US Public Health Service/US Department of Agriculture guidelines. Experimental protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee.

Guinea pigs were anesthetized under halothane vapors, and blood was obtained via heart puncture. Plasma samples

were collected, and preservation cocktail was added (aprotonin, 0.5 ml/100 ml; phenyl methyl sulfonyl fluoride, 0.1 ml/100 ml; and sodium azide, 0.1 ml/100 ml). One milliliter of plasma from each animal was stored at −80°C until further analysis. Livers were harvested and promptly frozen in dry ice the day of the heart puncture. Livers were then stored at −80°C until RNA extraction.

2.4. Cholesterol ester transfer protein activity

Cholesterol ester transfer protein activity levels were determined in the plasma according to Ogawa and Fielding [20] following separation of plasma from red blood cells. Cholesterol ester transfer protein activity was calculated by measuring the mass transfer of CE between HDL and apoB-containing lipoproteins. Physiologic CETP activity was determined in triplicate for each guinea pig without inhibition of lecithin-cholesterol acyltransferase. Samples were incubated at 37°C for 6 h in a shaking water bath. Simultaneously, additional samples for each guinea pig were frozen at −80°C without incubation and this was used as the 0 for calculations. Plasma total and free cholesterol and total and free HDL-C were measured. Cholesterol ester transfer protein activity was measured as the free cholesterol decreased over 6 h. This method has been well standardized for guinea pig plasma [21].

2.5. HMG-CoA reductase activity

HMG-CoA reductase (EC 1.1.1.34) activity was measured in hepatic microsomes as previously reported [22]. Protein content was measured [23] prior to analysis. Microsomes were incubated with 50 μl of a solution containing 7.5 nmol (0.33 GBq/nmol) [3-^{14}C] HMG-CoA, 4.5 mmol glucose-6-phosphate, 3.6 mmol EDTA, 0.45 mmol NADP and 0.3 IU glucose-6-phosphate dehydrogenase. [^{3}H]Mevalonic acid (0.024 GBq) was added as a recovery standard. The reaction was stopped after 15 min with 10 mol/L HCl (0.025 ml/tube). An excess of mevalonic acid was added, and samples were incubated for another 30 min at 37°C to allow for the conversion of mevalonic acid to mevalonalactone. After incubation, microsomes were pelleted by centrifugation for 1 min at 1000×g. An aliquot of the supernatant (0.1 ml) was applied to silica gel TLC plates and developed with acetone/benzene (1:1, v/v). The area containing the mevalonate (R_f=0.6–0.9) was scraped and mixed with 5 ml aquasol. Radioactivity was measured using a liquid scintillation counter (Packard, Downers Grove, IL).

2.6. Cholesterol 7α-hydroxylase activity

Cholesterol 7α-hydroxylase (EC 1.14.13.7) activity was assayed according to Jelinek et al. [4]. [^{14}C]Cholesterol was used as a substrate and delivered as cholesterol-phosphatidylcholine liposomes (1:8 by weight). After preparation by sonification, an NADPH-regenerating system (glucose-6-phosphate dehydrogenase, NADP and glucose-6-phosphate) was included as a source of NADPH. After addition of glucose-6-phosphate dehydrogenase (0.3 IU), samples were incubated for an additional 30 min. The reaction was stopped by the addition of 5 ml of chloroform–methanol 2:1 and 1 ml acidified water (0.05% sulfuric acid). Tubes were mixed, the top layer was discarded and samples were dried under nitrogen. Samples and 7α- and 7β-hydroxycholesterol standards were dissolved in 100 μl of chloroform, applied to silica gel TLC plates and developed with ethyl acetate–toluene (3:2). The plate was placed in iodine vapors to mark the 7α- and 7β-hydroxycholesterol standards and placed on XAR-5 film overnight. With the film as a guide, the location of the [^{14}C] 7α-hydroxycholesterol spots was determined, scraped from the plate and counted in a liquid scintillation counter.

2.7. Fecal bile acids

Fecal bile acids were assayed by a colorimetric method [24]. Briefly, feces were weighed, dried for 5 h at 37°C, pulverized and weighed again. Four milliliters of *t*-butanol/water (1:1 by v/v) was added to 0.2 g of fecal samples and heated at 37°C for 15 min with continuous agitation. The samples were then centrifuged at 3000 rpm for 10 min (JA-20 rotor in a J2-21 centrifuge, Beckman Coulter), and the supernatant was removed and the pellet was discarded. Each sample (200-μl aliquot) was analyzed in duplicate, and a blank reagent was added to a third aliquot to correct for the color provided by each sample. Samples were incubated at 37°C for 5 min. The reaction was stopped, and the color was read in a spectrophotometer at 530 nm. The amount of fecal bile acids was calculated as millimole per kilogram per day after subtracting the blank.

2.8. RNA extraction

Total RNA was extracted from liver using Trizol reagent. Briefly, 60 mg of liver was homogenized in 1 ml Trizol and incubated for 5 min at 15–30°C. Chloroform was added, and samples were shaken vigorously and incubated at 15–30°C for 2–3 min. Samples were centrifuged at 10,000 rpm for 15 min at 4°C. The aqueous phase was mixed with isopropyl alcohol, incubated at 15–30°C for 10 min and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was removed, and the pellet was mixed with 75% ethanol and centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant was removed, and the RNA pellet was air dried for ~10 min. RNA was resuspended in diethyl pyrocarbonate-treated water, quantified by absorbance at 260 nm and frozen into aliquots at −80°C.

2.9. RNA quantification

Cholesterol ester transfer protein and HMG-CoA reductase mRNA abundance were determined from representative samples (n=4 per group) using the Qiagen One-Step RT-PCR kit. RT-PCR was carried out in a Perkin Elmer Cetus DNA Thermal Cycler. With the exception of β-actin, all oligonu-

cleotide primers used for amplification were designed by Rea et al. [25] and were based on regions of high homology in the published rat [26] and human [27] sequences: CETP: 5′ primer, 5′-ATGGAATTCACACCATCTCCAACATCA-TGGC-3′, and 3′ primer, 5′-TTCTGGCAGGAGATCTTG-GGC-3′; for HMG-CoA-R: 5′ primer, 5′-ATGGAATTCC ATGGCTGGGAGCATAGGAG-3′, and 3′ primer, 5′-TCC-TTGAACACCTAGCATCTGC-3′; for β-actin: 5′ primer, 5′-AAGGACCTCTATGCCAACACAG-3′, and 3′ primer, 5′-GTACTCCTGCTTGCTGATCCAC-3′. The approximate size of each reaction product is as follows: CETP: 445 bp, HMG-CoA reductase: 250 bp, and β-actin: 220 bp. β-Actin was used as a control in all reactions. The reaction mixture contained 0.5 μg of total RNA. Amplification was carried out at an annealing temperature of 60°C for 27 cycles for CETP, 60°C for 31 cycles for HMG-CoA reductase and 60°C for 16 cycles for β-actin. Ten microliters of product was then size fractionated in a 1% agarose gel, and bands were visualized via ethidium bromide staining. Products were quantified by measuring the relative band intensity using Quantity One software (Bio-Rad Laboratories, Hercules, CA). Band intensities were corrected based on the β-actin signal for each sample.

2.10. Statistical analysis

Data were analyzed by one-way ANOVA. The Newman–Keuls test was used as a post hoc test. P values <.05 were considered statistically significant. All data were evaluated using SPSS version 11.0 (SPSS, Chicago, IL).

3. Results

The amount of food that the guinea pigs consumed was calculated by weighing food intake for 48 h 1 week prior to sacrifice. Food consumption was significantly elevated with ASBTi treatment compared to the control (P<.01). In contrast, food consumption was decreased significantly with COMBO treatment compared to the control (P<.01).

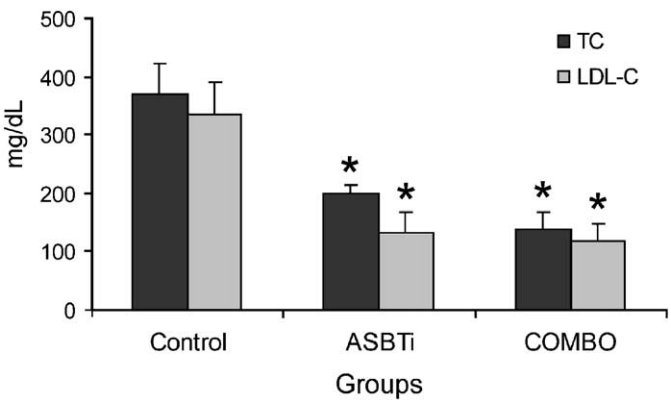

Fig. 1. Plasma total and LDL cholesterol concentrations in guinea pigs (n=4) used to analyze CETP and HMG-CoA reductase mRNA expression. *P<.05.

Table 1
Activities of CETP, HMG-CoA reductase and CYP7 of guinea pigs treated with 0% (control), 0.01% or 0.03% ASBTi and 0.05% simvastatin (COMBO)[a]

	CETP [μmol/(L h)]	HMG-CoA reductase [pmol/(min mg)]	CYP7 [pmol/(min mg)]
Control	50.0±10.2[a]	1.96±0.63[a]	5.28±1.4[a]
ASBTi	32.9±8.0[b]	4.26±1.03[c]	11.70±3.4[c]
COMBO	22.23±3.0[b]	3.45±0.43[c]	12.39±5.0[c]

[a] Data are presented as mean±S.D. for 10 guinea pigs. Values in the same column with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keuls as post hoc test.

Discrepancies in food consumption among groups are due to the taste of the different diets associated with the tested drugs. Based on the average food consumption for each group and the percentage of SC-435 and simvastatin known to be in each diet, the amount of drug consumed was calculated to be 0 and 47.7 mg/kg per day SC-435 for guinea pigs fed the control and ASBTi in treatments, respectively, and 13.4 mg/kg per day SC-435 and 21.4 mg/kg per day simvastatin for guinea pigs given COMBO treatment [1]. In addition, guinea pigs taking the ASBTi treatment weighed approximately 10% more than controls (P<.01), while guinea pigs taking the COMBO treatment weighed approximately 15% less than the controls (P<.01) [1]. To ensure that differences in weight and cholesterol intake did not influence plasma lipid concentrations, weight loss and cholesterol consumption were analyzed as covariates for measurements of plasma total, LDL-C, HDL-C and TG concentrations. Differences in body weight and dietary cholesterol intake did not influence plasma lipid levels (P>.05) (data not shown).

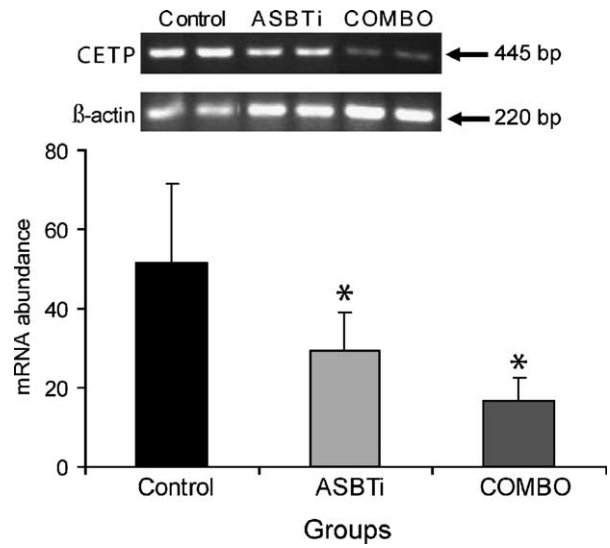

Fig. 2. Upper panel: Representative samples of CETP mRNA after control, ASBTi and COMBO treatments as measured by RT-PCR. Beta-actin was used as the control in all reactions. Lower panel: Mean mRNA levels for control, ASBTi and COMBO groups (n=4). *P<.05.

Fig. 1 shows the plasma total and LDL-C levels for the subset of guinea pigs used to analyze CETP and HMG-CoA reductase mRNA expressions ($P<.05$). Within this subset, plasma TC was reduced by 46% and 62% with ASBTi and COMBO treatments, respectively, compared to the control. In addition, plasma LDL-C was reduced by 40% and 64% in the ASBTi and COMBO subset groups, respectively ($P<.05$). Plasma CETP activity was 34% and 56% lower with ASBTi and COMBO treatments, respectively ($P<.05$) (Table 1). Similarly, CETP mRNA expression was reduced by 36% and 73% in ASBTi and COMBO groups compared to the control ($P<.05$), as shown in Fig. 2.

3.1. Hepatic lipids

As previously reported [1], hepatic TC was reduced by 60% with both ASBTi and COMBO treatments ($P<.01$), which was associated with a 60–80% lower hepatic free cholesterol ($P<.01$). In addition, hepatic TG was significantly reduced by 79% and 66% with ASBTi and COMBO treatments, respectively ($P<.01$). The concentration of hepatic esterified cholesterol was not different among groups ($P>.05$).

3.2. HMG-CoA reductase

HMG-CoA reductase activity was increased 2.17-fold and 1.76-fold with ASBTi and COMBO treatments ($P<.001$), respectively (Table 1). In agreement, HMG-CoA reductase mRNA expression was increased ~33% with ASBTi treatment compared to the control ($P<.01$) (Fig. 3). However, HMG-CoA reductase mRNA expression was not significantly altered with COMBO treatment ($P=.13$), suggesting that the regulation may be independent of changes in mRNA levels with the lower dose of SC-435 and simvastatin.

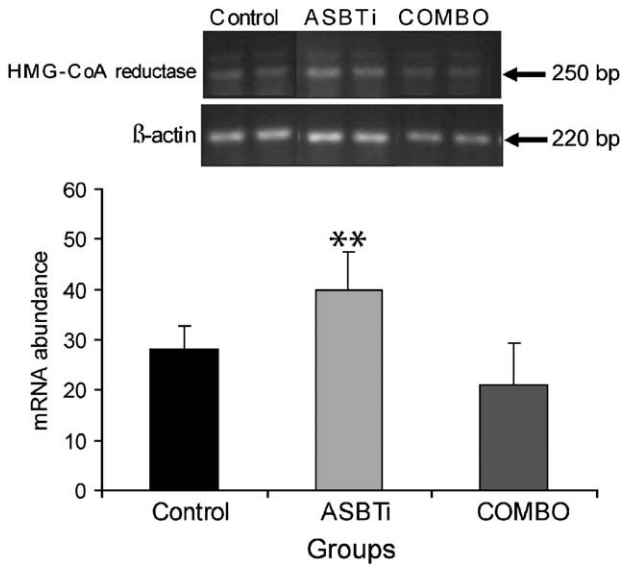

Fig. 3. Upper panel: Representative samples of HMG-CoA reductase mRNA after control, ASBTi and COMBO treatments as measured by RT-PCR. Beta-actin was used as the control in all reactions. The arrow points out the HMG-CoA reductase band. Lower panel: Mean mRNA levels for control, ASBTi and COMBO groups ($n=4$). **$P<.01$.

3.3. Bile acids and CYP7

Bile acid excretion was significantly increased by ASBTi and COMBO treatments. Bile acid values were 0.77 ± 0.51, 2.20 ± 0.68 and 4.61 ± 2.01 mmol/kg per day in the control, ASBTi and COMBO groups, respectively. Bile acid excretion was increased approximately twofold and fivefold by ASBTi and COMBO, respectively, compared to the control group. In agreement with these results, CYP7 activity was increased approximately twofold with ASBTi and COMBO treatments ($P<.001$) (Table 1).

4. Discussion

In our previous reports, we clearly demonstrated that guinea pigs fed hypercholesterolemic diets in combination with SC-435 for 12 weeks presented significant reductions in LDL-C and cholesterol concentrations in the aortic arch compared to animals fed a control diet [1]. This suggests that, in addition to the observed decreases in hepatic cholesterol via catabolism to bile acids, there may be other mechanisms associated with the hypocholesterolemic effects of SC-435. In the current report, significant increases in CYP7 activity and up-regulation of HMG-CoA reductase by SC-435 indicate alterations in hepatic cholesterol homeostasis due to the action of the drug in disrupting the enterohepatic circulation of bile acids. In addition, the observed decrease in both plasma activity and hepatic expression of CETP indicates that as a result of the action of the drug the intravascular processing of lipoproteins is altered. Since high CETP has been associated with increases in plasma cholesterol and atherosclerosis in animal models [28], this is an important finding, which contributes to our knowledge on the hypocholesterolemic mechanisms of ASBT inhibitors.

4.1. Effects of the ASBT inhibitor on hepatic cholesterol homeostasis

One major function of the liver is to rid the body of excess cholesterol by converting cholesterol to bile acids via CYP7. A previous study in our laboratory showed a 30% increase in CYP7 activity in guinea pigs treated with 13.4 mg/kg per day SC-435 [9]. Furthermore, significant increases in CYP7 activity and expression have been reported in miniature pigs treated with 10 mg/kg per day SC-435 or a combination of 5 mg/kg per day SC-435 plus 3 mg/kg per day atorvastatin [15,29]. In agreement, our current study showed a twofold increase in CYP7 activity with the ASBTi and COMBO treatments. Moreover, fecal bile acids in these guinea pigs increased two- and fivefold with ASBTi and COMBO treatments, respectively. Also, ASBTi and COMBO treatments resulted in significant reductions in hepatic free cholesterol concentrations, which

is the substrate for bile acid synthesis. These results suggest that the decrease in bile acid absorption, brought about by ASBT inhibition, led to an induction of CYP7 activity. Overall, the increases in CYP7 activity and bile acid excretion, as seen with ASBTi and COMBO treatments, are a major mechanism, which may partially account for the hypocholesterolemic effect of SC-435.

To further study the compensatory mechanisms for maintaining hepatic cholesterol levels, we measured hepatic HMG-CoA reductase activity and mRNA expression in guinea pigs treated with SC-435. We found that ASBTi treatment significantly increased both reductase activity and expression, leading to an increase in cholesterol biosynthesis. Our findings are in agreement with reported increases in hepatic reductase activity and mRNA expression in miniature pigs treated with SC-435 [30]. In addition, significant increases in hepatic HMG-CoA reductase mRNA levels have also been observed in apoE−/− mice on SC-435 treatment [15]. Overall, the ability of HMG-CoA reductase to respond to decreases in hepatic cholesterol levels is one mechanism by which cholesterol concentrations are maintained in the liver.

HMG-CoA reductase activity was also increased with COMBO treatment, suggesting that this increase was primarily due to the effects of SC-435. While activity was markedly increased with COMBO treatment, HMG-CoA reductase mRNA expression was not different from the controls. In contrast to our findings, Telford et al. [29] reported a 2.5-fold increase in hepatic HMG-CoA reductase mRNA levels in miniature pigs fed a combination of SC-435 and atorvastatin. However, previous studies in our laboratory have shown that simvastatin has a modest effect on HMG-CoA reductase mRNA expression in guinea pigs, which is much lower compared to the effects induced by atorvastatin in this animal [22]. These data suggest that atorvastatin and simvastatin may have different half lives, which may partially explain the variation between studies. Furthermore, the rate of degradation of hepatic HMG-CoA reductase appears to be reduced by either sterols or nonsterols in the presence of cholesterol-lowering drugs [31]. A decrease in the rate of reductase degradation may explain why HMG-CoA reductase mRNA levels were not affected by the COMBO treatment, despite the significant increase in activity.

4.2. Effects of the ASBT inhibitor on intravascular processing of lipoproteins

In addition to hepatic cholesterol metabolism, it is important to analyze lipoprotein remodeling and composition to better understand the mechanisms responsible for the cholesterol-lowering effects of SC-435. Cholesterol ester transfer protein promotes the redistribution of CE and TG between HDL particles and apoB-containing lipoproteins. Although there is controversy regarding the atherogenic role of CETP, serum CETP activity levels are positively associated with VLDL-C and LDL-C levels in both rabbits [32] and humans [33]. Likewise, the decreased CETP activity levels seen with ASBTi and COMBO treatments coincide with the significant reductions in VLDL-C, LDL-C and the cholesterol concentrations in the aortic arch observed in these treatment groups [1].

Cholesterol ester transfer protein increases the CE content and therefore the atherogenicity of VLDL and LDL particles [13]. Studies have shown that reductions in LDL CE concentrations are related to faster catabolic rates in plasma [34]. Our previous report shows that guinea pigs treated with ASBTi and COMBO had significantly lower LDL CE concentrations compared to controls [1]. The lower CETP activity and mRNA expression may partially explain the lower concentrations of LDL CE previously found with SC-435 treatment.

In the current study, we demonstrated that significant reductions in plasma LDL-C concentrations were related to alterations in the enterohepatic circulation of bile acids by ASBT inhibition, which subsequently resulted in modulation of the activity and mRNA expression of important enzymes of hepatic cholesterol homeostasis. In addition, the changes in lipoprotein compositions, due to altered CETP activity and mRNA expression, could add to the antiatherogenic potential of SC-435 treatment in guinea pigs.

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