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Effect of Ezetimibe on some biochemical factors and expression of Intestinal Scavenger receptor class B type I (SR-BI) in obsess mouse

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Abstract

Ezetimibe is a new and very effective drug which reduced cholesterol. Ezetimibe well tolerated by the patients that selectively blocks cholesterol absorption from intestine.In intestine scavenger receptor class B, type I (SR-BI) has recognized as a cholesterol and triglyceride transporter.In this experiment weexamined the effect of ezetimibe on lipid profile, glucose levels as well as SR-BI expression in intestine of hypercholesterolemic mice. Mice randomly divided into three groups (n=8); group 1: hypercholesterolemic, group 2: ezetimibe and group 3: chow only. After one-month mice were sacrificed, biochemical factors were determined enzymatically as well as the levels of SR-BImRNA and protein were determined by RT-PCR and westernblotrespectively. Compared with hypercholesterolemic control, ezetimibe significantly decreased low-density lipoprotein cholesterol (LDL-C) (P<0.05) and total cholesterol (P<0. 05). Intestinal SR-BI mRNA and protein were significantly decreased in intestine by ezetimibe (P<0.05). Taken together, ezetimibe significantly reduced total cholesterol as well as led to down-regulation of SR-BI in mouse intestine.

Keywords: Ezetimibe, LDL-C, cholesterol, SR-BI, mouse

Introduction

Heart disease is the major killer in the world. Many risk factors including low level of high-density lipoprotein cholesterol (HLD-C) and high levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG) have been powerfully associated with greater cardiovascular disease prevalence[1-3].

Ezetimibe is a new hypolipidemicdrugthat inhibits the intestinal absorption of cholesterol. This drug is well tolerated generally, and its side effects are similar to placebo[4]. Ezetimibe with block of sterol transporter Niemann–Pick C1-like 1 (NPC1L1) protein inhibits absorption of cholesterol up to 96% in animal models and nearly 50% in patients with mild hypercholesterolemia[5].

Many proteins such as NPC1L1, scavenger receptor class B, type I (SR-BI) and ATP binding cassette family G5 and G8 (ABCG5 and ABCG8) have key role in cholesterol and sterol transporter in intestine[6]. SR-BI selectively transports cholesterol esters (CE) and other lipids from HDL to liver cells. Studies have showed that homozygous null SR-BI KO mice have high cholesterol levels with large HDL particles in their serum[7]. In this experiment, we tested the influence of ezetimibe on lipid profile, glucose and also examined the effect of this combination on SR-BI protein in obsess mice.

Material and Methods

Animals and treatments: Male N-Mary mice were maintained at 21ºC in 12h light/12h dark cycleandapproximately 60% humidity. Following acclimatization for one week, mice were randomly separated into 3 groups (8 mice in every group): group 1. Chow + 2% cholesterol + 0.5% cholic acid, group, group 2: chow + 0.005% (w/w) Ezetimibe + 2% cholesterol + 0.5%cholic acid, and group 3: chow only.

Blood glucose, cholesterol and triglyceride were at the baseline before treatment and there were not different among mice. Animals were checked daily and body weight was recorded every 48 hours. Ezetimibe was dissolved in corn oil and mixed with diet. After 1 month fasting mice were anesthetized and sacrificed. Blood was collected from heart, and intestine was removed, washed with PBS, and stored in -70°C till use[8-11]. All process of this experiment has been permitted by the Animal Research Ethic Committee of Kerman University, Kerman, Iran.

Biochemical factors: Serum was achieved by blood centrifugation for 10 minutes at 3000g and then kept at -20°C until analyze. The levels of Fasting blood glucose, HDL-C, cholesterol, triglyceride and ALT, AST, and GGT were measured enzymatically. The levels of LDL-C and VLDL-C were calculated using Friedwald equation[9-11].

RT-PCR: Isolated enterocytes were instantly extracted with Trizol Reagent (Bioneer, Korea) according to the manufacturers' procedure. The cDNA Synthesis was performed according to the manufacturer's instructions (Fermentas, Lithuania). For PCR reaction, 35 cycles of PCR amplification were achieved with denaturation at 95 ºC for 30s, annealing at 63 ºC for 30s, and extension at 72ºC for 30s by PCR machine. The yields electrophoresed on a 2.5% agarose gel and visualized by staining with ethidium bromide[11-14]. The following primers were used in this study. Mouse SR-BI primer; forward (F): 5'-CAC CTT CAA TGA CAA CGA CAC C-3'and reverse (R): 5'-TCT CTG AGC CAT GCG ACT TG-3'. Mouse Beta actin Primer; F: 5'-TGG AAT CCT GTG GCA TCC ATG AAA C-3' and R: 5'-TAA AAC GCA GCT CAG TAA CAG TCC G-3'.

Western blotting: Protein extracts from small intestine (120 µg), were separated on a 12.5% SDS-PAGE gel and transferred to a PVDF (Roche Applied Science) membrane. The membrane probed with SR–BI (1:2500 dilutions, Novus Biological) and β-Actin (1:2500 dilutions, Novus Biological) antibodies. Subsequently, membrane incubated with a secondary peroxidase-conjugated anti body and protein signals were pictured via chemi luminescence (Roche Applied Science). The densities of bands were determined with Lab Work analyzing software (UVP, UK). Data are expressed as the percent ratio of SR–BI to *β*-Actin[10,11,13].

Statistical analysis: Analyses of this experiment were completed with SPSS 14.0 for Windows (SPSS Inc., Chicago, USA). All data are offered as mean ± SEM. Differences among the groups were evaluated by one-way analysis of variance with ANOVA (Tukey). Different in groups were considered significant when P was less than 0.05.

Results and Discussion

Body weight, Lipid profiles and blood sugar in different groups are show in table-1. Cholesterol, LDL-C, triglyceride, VLDL-C, ALT, AST, and GGT markedly increased in animals fed atherogenic diet compared to chow diet. Body weight did not showed significant differences between ezetimibe and hypercholesterolemic control. However, weight gain was markedly high in hypercholesterolemic group as compared to chow. Serum cholesterol, LDL-C, ALT, AST, and GGT significantly decreased in ezetimibe-treated group compared to hypercholesterolemic mice. Triglyceride and VLDL-Cnon-significantly decreased in ezetimibe-treated group compared to hypercholesterolemic mice.

Effect of ezetimibe on gene expression: RT-PCR products of SR-BI showed projected band of 82 bp. Intestinal SR-BI mRNA significantly reduced in ezetimibe compared to hypercholesterolemic animals (P<0.05) (figure-1). Immunoblot analysis of the intestine protein probed with anti SR-BI revealed bands with expected sizes of 82 KD. SR-BI protein significantly reduced in ezetimibe treated animal compared to hypercholesterolemic mice (P<0.05) (figure-2).

Table-1
Comparison of biochemical factors among different groups

Biochemical factors	Control	Ezetimibe	Chow
Body weight (g)	37.5 ± 1.5[e]	35.9 ± 0.9	30.7 ± 0.7
FBS (mg/dl)	160.1 ± 5.6	148.2 ± 12.1	138.1 ± 9.7
TC(mg/dl)	230.1 ± 4.5[f]	180.2 ± 6.6[b]	129 ± 13.4
TG (mg/dl)	160.5 ± 7.5[e]	144.2 ± 4.1	133.5 ± 4.0
VLDL-C (mg/dl)	32.1 ± 1.5[e]	28.5 ± 0.8	26.7 ± 0.8
HDL-C(mg/dl)	110.2 ± 8.1	95.0 ± 8.4	87.4 ± 9.7
LDL-C (mg/dl)	92.8 ± 9.8[f]	52.6 ± 10.1[a]	24.9 ± 5.5
AST (u/l)	55.8 ± 5.1[a]	43.5 ± 4.7[a]	41.5 ± 4.7
ALT (u/l)	58.4 ± 4.3[a]	45.1 ± 3.6[a]	43.1 ± 5.2
GGT (u/l)	56.8 ± 5.1[a]	42.5 ± 4.3[a]	38.9 ± 4.1

TC: Total cholesterol, TG: Triglyceride, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, ALT: alanine amino transferase, AST: aspartate amino transferase, GGT: Gamma glutamyl transferase. Data represent as mean ± SEM (n = 8), [a]p< 0.05 compared with Hypercholestrolemic. [b]p< 0.01 compared with Hypercholestrolemic. [c]p< 0.001 compared with Hypercholesterolemic. [d]p< 0.05 compared with chow. [e]p< 0.01 compared with chow. [f]p< 0.001 compared with chow.

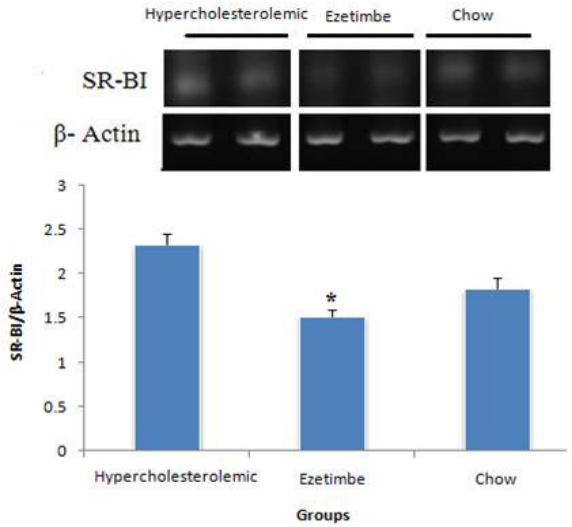

Figure-1
Reverse Transcription PCR of SR-BI in intestine of animals which treated differently. The average band density ratio of intestinal SR-BI mRNA in three groups including (n=8); hypercholesterolemic, ezetimibe and chow received. Data in the graph are represented as mean ± SEM. The density of intestinal SR-BI mRNA significantly reduced in the in the ezetimibe group. *P <0.05 between ezetimibe with chow and hypercholesterolemic groups

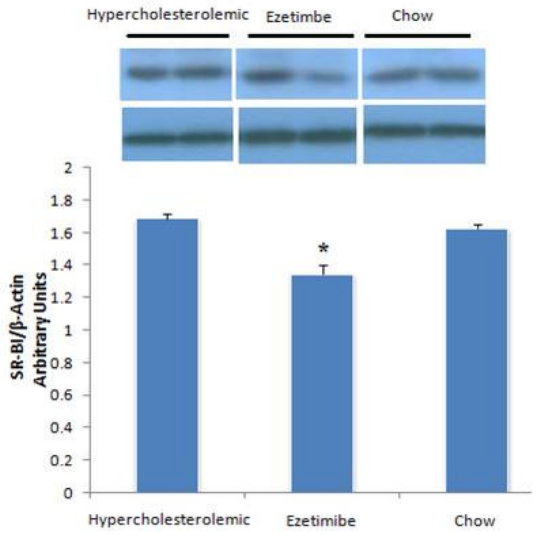

Figure-2

Westernblot of SR-BI proteinof SR-BI in intestine of animals which treated differently, The average band density ratio of intestinal SR-BI mRNA in three groups including (n=8); hypercholesterolemic, ezetimibe and chow received. Data in the graph are represented as mean ± SEM. The density of intestinal SR-BI protein significantly reduced in the in the ezetimibe group. *P <0.05 between ezetimibe with chow and hypercholesterolemic groups

Biochemical factors: In the present study we showed that atherogenic diet (2% cholesterol and 0.5% cholic acid) markedly increased Body weight, cholesterol, LDL-C, triglyceride and VLDL-C. Animal experiments reported that high cholesterol diet stimulated hypercholesterolemia and consequently atherosclerosis[15].Many human and animal studies have examined the hypolipidemic and hypocholesterolemic effects of ezetimibe. The serum total cholesterol (21.7%) andLDL-C (35%) levels were markedly reduced by ezetimibe compared with hypercholesterolemic control mice. Van Heek M et al. reported that administration of 1mg/ kg of ezetimibe in rat fed with cholesterol inhibited cholesterol absorption by 92-96% [16]. In Van Heek M et al. study combination of ezetimibe with a statin (atorvastatin and simvastatin, 10–80 mg/day and pravastatin and lovastatin, 10–40 mg/day) was evaluated, they showed that combination of ezetimibe with atorvastatin led to 50-60%, simvastatin, 44-57%, pravastatin, 34-41% and lovastatin, 33-45% reduction in LDL-C levels[4].

Oxidation of LDL-C is known as a basic process in the atherosclerosis pathogenesis since it contributes to formation of foam, dysfunction of endothelial, and inflammation. Results of many experiments have revealed that oxidised form of cholesterol in the diet raise the atherosclerosis development. A recent experiment reported that ezetimibe when administered following a diet containing oxidised cholesterol can decline oxysterols by 50% in serum[17]. A mixture of external[18-23] and internal[24]antioxidants protected body against oxidative stress attack.

Bays et al. (2001) and Dujovne et al. (2002) showed that ezetimibe decrease TG levels by 1.7 to 9.4%, but this decrease are not always significant. In our study, ezetimibe decrease triglyceride levels by but it was not significant[25].

Ezetimibe is selectively inhibitedintestinal cholesterol absorption with blockage of the sterol transporter NPC1L1 protein[25]. In this study SR-BI protein and mRNA were significantly reduced in the intestine by ezetimibe. The result of Hauser H et al. study showed that SR-BI has key role in absorption of cholesteryl esters[26]. The ability of ezetimibe to inhibit of SR-BI and consequently plasma cholesterol suggests that SR-BI has vital role in cholesterol absorption. Altmann SW et al. also revealed that SR-BI is one of the main intestinal cholesterol transporters and can be inhibited by SCH354909 (an ezetimibe derivative)[27].These results also were agreement with During A et al. result which showed that ezetimibe markedly decreased SR-BI in Caco-2Cells[28]. Recently Bietrix et al. with generate of transgenic mice over-expressed SR-BI, demonstrated that this transporter has chief role in cholesterol and triglyceride absorption[29]. We found that ezetimibe inhibited of mRNA and protein of SR-BI in the intestine, leads to reduction of plasma cholesterol and LDL-C.

Conclusion

In conclusion, we suggest that ezetimibe is a new medicine which significantly reduces lipids s and potentially can be a good option for treatment of hyperlipidemia and diabetes. Ezetimibe also lead to inhibition of SR-BI in the intestine which has an important effect in reduction of total cholesterol and LDL-C.

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