Biochemical and Biophysical Research Communications 463 (2015) 48–53

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Spontaneous and diet-aggravated hemolysis and its correction by probucol in SR-BI knockout mice with LDL-R deficiency

Jiawei Liao [a], Mingming Gao [a], Mengyu Wang [a], Xin Guo [a], Wei Huang [a, *], George Liu [a, b, *]

[a] Institute of Cardiovascular Sciences and Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Peking University Health Science Center, Beijing 100191, China
[b] Center for Molecular and Translational Medicine, Peking University Health Science Center, Beijing 100191, China

ARTICLE INFO

Article history:
Received 25 April 2015
Accepted 6 May 2015
Available online 15 May 2015

Keywords:
Hemolysis
SR-BI
Free cholesterol
Probucol

ABSTRACT

Background: High density lipoprotein receptor SR-BI plays a vital role in cholesterol homeostasis. Depletion of SR-BI causes plasma free cholesterol (FC) accumulation, which disrupts erythrocytes membrane and might induce hemolytic anemia. Here we explored the effects of hypercholesteremia, induced by depletion of low density lipoprotein receptor (LDL-R) and high fat diet (HFD) feeding, on plasma FC and possible hemolysis in SR-BI knockout (KO) mice, and the therapeutic effects of a lipid-lowering drug probucol.

Methods and results: To determine the effects of LDL-R depletion, SR-BI KO mice were cross-bred with LDL-R KO mice to generate SR-BI/LDL-R double KO (dKO) mice. Compared to control wild type (WT), SR-BI KO and LDL-R KO mice fed normal chow diet (NCD), dKO mice fed NCD had increased plasma FC and developed macrocytic anemia, splenomegaly, jaundice and renal tubular hemosiderin deposition, indicating spontaneous hemolysis. To determine the effects of HFD feeding and probucol therapy, dKO and LDL-R KO mice were fed HFD containing 0.5% cholesterol and 20% fat with or without 1% probucol. HFD further increased plasma FC and aggravated hemolysis while probucol almost normalized plasma FC and corrected hemolysis in dKO mice.

Conclusion: We demonstrated that in SR-BI KO mice, hypercholesteremia due to LDL-R deficiency significantly increased plasma FC and induced spontaneous hemolysis, which could be further exacerbated by HFD feeding. Probucol almost normalized plasma FC and corrected diet-aggravated hemolysis in SR-BI KO mice with LDL-R deficiency.

1. Introduction

Scavenger receptor class B type I (SR-BI) is the major high density lipoprotein (HDL) receptor in mammalians. Mainly expressed in the liver and steroidogenic organs such as the adrenal glands, the ovaries and the testis, it mediates the selective uptake of cholesterol esters (CEs) in the HDLs, thus playing a vital role in reverse cholesterol transport (RCT) and global cholesterol homeostasis. Defect in SR-BI causes disrupted RCT and thus cholesterol enrichment, mainly as the form of free cholesterol (FC), in the HDLs, contributing to HDL dysfunctions [1–3].

Circulating in the bloodstream, the erythrocytes exchange lipids directly with lipoproteins via the erythrocyte membrane in nonspecific mechanisms [4]. Abnormalities in lipoproteins might affect the lipids content and thus the properties of the erythrocyte membrane. A previous study has already demonstrated that due to increased FC in the HDLs, cholesterol accumulated in the erythrocyte membrane of SR-BI knockout (KO) mice, leading to decreased membrane deformability and osmotic fragility [5]. The changes in the erythrocyte membrane might cause accelerated erythrocytes degradation and induce hemolysis. Here we explored the effects of hypercholesteremia, induced by depletion of low density lipoprotein receptor (LDL-R) and high fat diet (HFD) feeding, on the plasma FC and possible hemolysis in SR-BI KO mice, and the therapeutic effects of a lipid-lowering drug probucol.

* Corresponding authors. Institute of Cardiovascular Sciences and Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Peking University Health Science Center, Beijing 100191, China.
E-mail addresses: huangwei@bjmu.edu.cn (W. Huang), georgeliu@bjmu.edu.cn (G. Liu).

http://dx.doi.org/10.1016/j.bbrc.2015.05.015
0006-291X/

2. Material and methods

2.1. Animals and diets

To determine the effects of hypercholesterolemia induced by LDL-R deficiency in SR-BI KO mice, SR-BI/LDL-R double KO (dKO) mice and control wild type (WT), SR-BI KO and LDL-R KO mice were fed normal chow diet (NCD) all the way until there were 5 months old; To determine the effects of HFD feeding and probucol therapy, SR-BI/LDL-R dKO and control LDL-R KO mice of 10–12 weeks old were fed a HFD containing 0.5% cholesterol (AMRESCO, USA) and 20% fat with or without 1% probucol (Natural-Med, USA) for 12 weeks. WT, SR-BI KO and LDL-R KO mice were supplied by Peking University Experimental Animal Center while SR-BI/LDL-R dKO mice were generated by cross-breeding SR-BI KO with LDL-R KO mice. All the mice included in the experiments were females. The housing, care and all the experimental procedures were conducted following the regulations of the National Institute of Health and approved by Animal Care Committee at Peking University.

2.2. Plasma lipids analysis

After the mice were fasted for 4 h, blood samples were drawn into tubes coated with heparin by retro-orbital venous plexus puncture and centrifuged at 4000 rpm for 10 min to collect the plasma supernatant. The plasma total cholesterol (TC) and FC were measured using commercial kits (Applygen, China).

2.3. Erythrocyte parameters analysis

Blood samples were drawn by retro-orbital venous plexus puncture and collected in tubes with no anticoagulant. The bloods (20 μl) were then immediately diluted and blended in specialized dilution buffer (Nihon Kohden, Japan). Erythrocyte parameters were measured with an automated blood count analyzer (MEK-6318K; Nihon Kohden, Japan).

2.4. Organ gravimetric and histological analysis

Mice were weighed, euthanized and flushed with 20 ml 0.01M phosphate buffer solution through the left ventricle. The spleens and kidneys were weighed, fixed in 4% paraformaldehyde solution for 4 h and then kept in 20% sucrose solution for overnight. Embedded in paraffin, the kidneys were cross-sectioned at 2 μm. Renal tubular hemosiderin deposition were visualized by Perl's Prussian blue staining.

2.5. Statistical analysis

Data were presented as mean ± SEM. Statistical significance was evaluated by one-way ANOVA and P value <0.05 was regarded as significant.

3. Results

3.1. LDL-R deficiency significantly increased plasma FC and led to spontaneous hemolysis in SR-BI KO mice

On NCD feeding, SR-BI depletion led to a roughly 1-fold and 4-fold increase of plasma TC and FC respectively, resulting in a roughly 1.5-fold increase of plasma FC/TC in WT mice (Table 1). A previous study has demonstrated that the increased plasma FC in SR-BI KO mice led to cholesterol accumulation in the membrane of the erythrocytes, resulting in decreased membrane deformability and osmotic fragility and thus increased degradation of the erythrocytes [5]. Here we showed mice depleted of SR-BI had slightly decreased erythrocyte count and hemoglobin (HGB), and slightly enlarged mean corpuscular volume (MCV) and decreased mean corpuscular hemoglobin concentration (MCHC) (Table 1), indicating no significant anemia in our female SR-BI KO mice. No significant enlargement of the spleen (Fig. 1A–B), no jaundice (Fig. 1C) and renal tubular hemosiderin deposition visualized by Perl's Prussian blue staining (Fig. 1D) were also observed in SR-BI KO mice. LDL-R depletion led to a roughly 1-fold increase of plasma TC and FC in both WT and SR-BI KO mice, however, as the increase of plasma TC and FC was proportional, there was no significant change of plasma FC/TC (Table 1). While hypercholesteremia due to LDL-R depletion failed to induce any significant change of the erythrocytes in WT mice, it caused a significant 26.77% decrease of erythrocyte count and 11.59% decrease of HGB together with a significant 26.76% increase of MCV and a slightly decrease of MCHC in SR-BI KO mice (Table 1). LDL-R depletion also led to a roughly 3-fold enlargement of the spleen (Fig. 1A–B) and the onset of jaundice (Fig. 1C) and renal tubular hemosiderin deposition (Fig. 1D) in SR-BI KO mice. When compared to WT mice, combined depletion of SR-BI and LDL-R led to a 33.46% decrease of erythrocyte count and 18.67% decrease of HGB together with a 31.51% increase of MCV and a 6.63% decrease of MCHC (Table 1), indicating the spontaneous onset of macrocytic anemia in SR-BI/LDL-R dKO mice. Macrocytic anemia,

Table 1
Analysis of the plasma cholesterol and erythrocytes in mice on NCD.

	WT (n = 6)	SR-BI KO (n = 5)	LDL-R KO (n = 11)	SR-BI/LDL-R dKO (n = 10)
Plasma lipids				
TC(mg/dl)	95.23 ± 4.642	199.9 ± 4.453,*	222.1 ± 9.577,###	406.8 ± 14.41, $$$; &&&
FC(mg/dl)	21.33 ± 2.233	95.35 ± 5.486,**	47.27 ± 2.484	211.8 ± 9.056, $$$; &&&
TC/FC	0.2220 ± 0.0162	0.4798 ± 0.0361,***	0.2134 ± 0.0082	0.5220 ± 0.0170, &&&
Erythocytes				
RBC Count(× 10^{12}/L)	8.978 ± 0.2990	8.158 ± 0.4530	8.825 ± 0.1700	5.974 ± 0.3680, $$$; &&&
HGB(g/L)	150.2 ± 3.219	138.0 ± 3.240	151.1 ± 1.654	122.0 ± 5.774, &&&
MCV(fL)	44.30 ± 0.2530	45.96 ± 0.8948	44.12 ± 0.1689	58.26 ± 1.640, $$$; &&&
MCHC(g/L)	380.2 ± 18.11	370.4 ± 6.990	360.7 ± 2.115	355.0 ± 6.493

Data were presented as mean ± SEM. RBC: red blood cell.
*: SR-BI KO vs WT.
#: LDL-R KO vs WT.
$: SR-BI/LDL-R dKO vs SR-BI KO.
&: SR-BI/LDL-R dKO vs WT.