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Reduction of Apolipoprotein E-rich HDL Levels with Berberine Stimulates Reverse Cholesterol Transport in Hyperlipidemic Hamsters Treated with CETP Inhibitor Torcetrapib

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INTRODUCTION

Cholesteryl ester transfer protein (CETP) inhibition increases the levels of enlarged/apolipoprotein E rich HDL particles (apoE-HDL). Whether these particles are functional to promote reverse cholesterol transport (RCT) remains unclear. Here we investigated this issue in hyperlipidemic hamsters.

METHODS

- Golden Syrian hamsters were fed a chow or a hyperlipidemic diet (27% fat, 0.5% cholesterol, 0.25% deoxycholate and 10% fructose in drinking water) over 4 weeks. Biochemical parameters were then measured to evaluate the effects of the hyperlipidemic diet.
- To evaluate the effects of CETP inhibition on reverse cholesterol transport, hyperlipidemic hamsters were treated with vehicle or CETP inhibitor torcetrapib 30mg/kg/day alone or in combination with berberine 150mg/kg/day over 14 days. Biochemical parameters were measured after 10 days of treatment.
- After 11 days of treatment, hamsters were injected i.v. with ^{3}H-cholesteryl oleate labeled oxidized LDL (^{3}H-oxLDL) to measure *in vivo* reverse cholesterol transport. ^{3}H-tracer reappearance was measured in plasma and HDL at time 24, 48 and 72 hours.
- Hamsters were then sacrificed after 72 hours and liver was harvested to measure hepatic ^{3}H-tracer recovery. Feces were collected over 72 hours to measure ^{3}H-radioactivity and mass in fecal cholesterol and bile acids fractions.

CONCLUSION AND PERSPECTIVES

- **CETP inhibition with torcetrapib significantly increases HDL-C and apoE-HDL levels but does not promote reverse cholesterol transport in hyperlipidemic hamsters.**
- **Combination of torcetrapib with berberine, a compound known to up-regulate LDL-receptor expression, reduces apoE-HDL levels and promotes reverse cholesterol transport in hyperlipidemic hamsters.**
- **Stimulating reverse cholesterol transport under CETP inhibition requires up-regulation of LDL-receptor. These findings should be investigated in humans to evaluate the benefits of CETP inhibitors.**

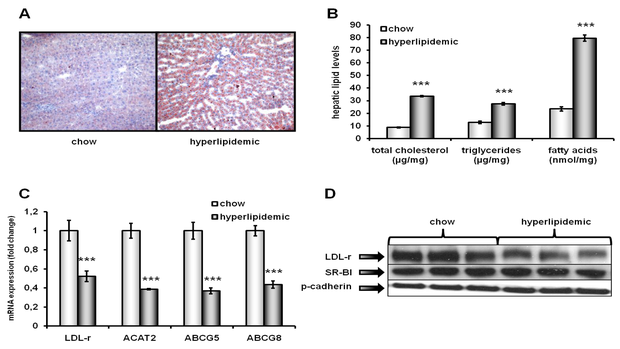
RESULTS

1. Effects of the hyperlipidemic diet on biochemical parameters

	chow	hyperlipidemic
blood glucose (mg/dL)	72 ± 3	116 ± 4***
plasma insulin (µU/mL)	5.7 ± 0.4	19.1 ± 2.5***
HOMA-IR (mM*µU/mL/22.5)	0.8 ± 0.1	5.4 ± 0.6***
triglycerides (g/L)	0.83 ± 0.07	3.87 ± 0.50***
total cholesterol (g/L)	1.24 ± 0.04	3.05 ± 0.12***
HDL-cholesterol (g/L)	0.84 ± 0.03	1.62 ± 0.09***
non HDL-cholesterol (g/L)	0.40 ± 0.02	1.43 ± 0.15***
HDL-cholesterol/total cholesterol	0.68 ± 0.01	0.54 ± 0.04***
CETP activity (pmol/µL/h)	52 ± 2	80 ± 2***

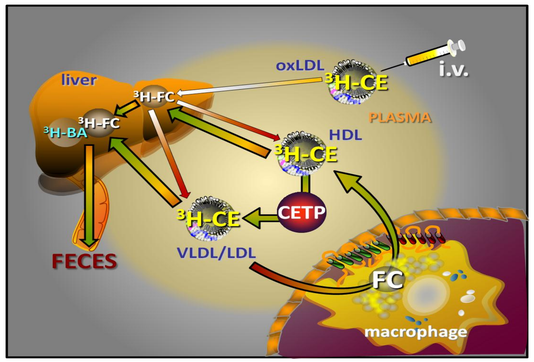
Biochemical parameters in hamsters after 4 weeks of chow or hyperlipidemic diet.
(***p<0.001 vs. vehicle)

2. Effects of the hyperlipidemic diet on liver parameters



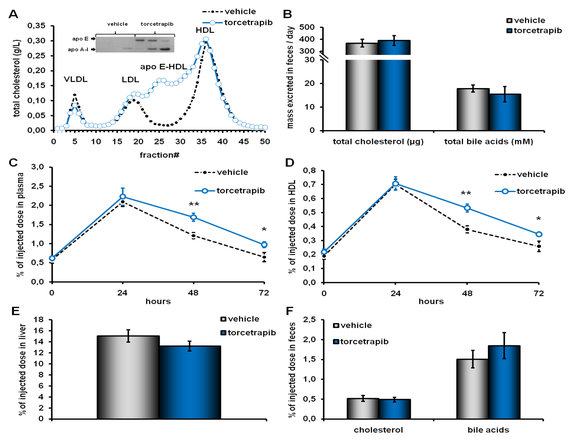
Liver Oil red O/hematoxylin staining (A), hepatic lipids levels (B), hepatic mRNA levels (C), and hepatic LDL-receptor/SR-BI protein expression in hamsters fed a chow or a hyperlipidemic diet for 4 weeks.
(***p<0.001 vs. chow)

3. Measurement of *in vivo* reverse cholesterol transport



Measurement of *in vivo* reverse cholesterol transport using ^{3}H-cholesteryl oleate labeled oxidized LDL.
(BA, bile acids; CE, cholesteryl esters; CETP, cholesteryl ester transfer protein; FC, free cholesterol)

4. Torcetrapib does not promote reverse cholesterol transport in hyperlipidemic hamsters



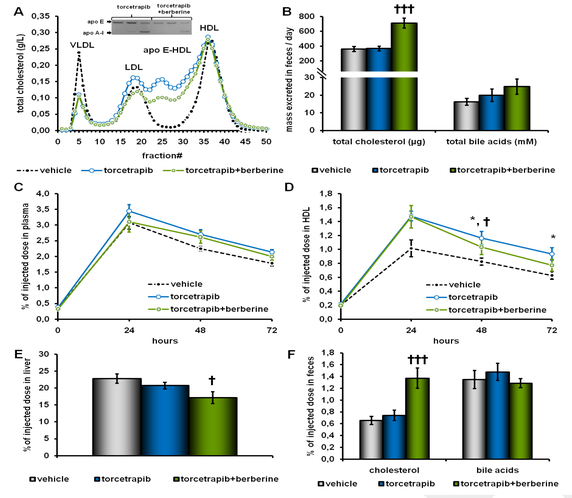
Fast Protein Liquid Chromatography profiles (A), fecal cholesterol/bile acids mass excretion (B), ^{3}H-tracer recovery in plasma (C), HDL (D), liver (E) and feces (F) after injection of ^{3}H-cholesteryl oleate labeled oxidized LDL in hyperlipidemic hamsters treated with vehicle or torcetrapib 30mg/kg.
(*p<0.05, **p<0.01 vs. vehicle)

5. Effects of torcetrapib and torcetrapib+berberine on biochemical parameters

	vehicle	torcetrapib	torcetrapib + berberine
Total cholesterol (g/L)	3.80 ± 0.12	5.15 ± 0.27***	4.43 ± 0.20
HDL-cholesterol (g/L)	2.04 ± 0.13	2.63 ± 0.13**	2.28 ± 0.10
Triglycerides (g/L)	3.02 ± 0.24	1.20 ± 0.09***	1.20 ± 0.13†††
CETP activity (pmol/µL/h)	58 ± 7	40 ± 2***	43 ± 3†††
Liver mass (g)	5.4 ± 0.3	5.8 ± 0.1	4.9 ± 0.2
Liver cholesterol (mg/g)	46.0 ± 2.6	47.2 ± 4.2	34.8 ± 2.5†
Liver triglycerides (mg/g)	33.1 ± 3.0	30.1 ± 1.1	32.8 ± 2.1

Plasma and liver parameters in hyperlipidemic hamsters treated with vehicle, torcetrapib 30mg/kg or torcetrapib 30mg/kg + berberine 150mg/kg.
(**p<0.01, ***p<0.001 vs. vehicle; †p<0.05, † † †p<0.001 vs. vehicle)

6. Torcetrapib + berberine combination promotes reverse cholesterol transport in hyperlipidemic hamsters



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Authors disclosure: F. Briand, Q. Thieblemont, E. Muzotte and T. Sulpice are employees of Physiogenex.

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