HUMAN APOLIPOPROTEIN C1 TRANSGENESIS TO INHIBIT CETP AND TO ATTENUATE ATHEROSCLEROSIS IN HYPERCHOLESTEROLEMIC RABBITS

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OBJECTIVE

Apolipoprotein (apo) C1 is a 6.6 kDa protein associated with HDL and VLDL. ApoC1 alters triglyceride clearance by inhibiting lipolysis and VLDL uptake, and it also favors cholesterol accumulation in HDL, especially by inhibiting CETP. Apart from studies in mice, which lack CETP, the impact of apoC1 on atherosclerosis in animal models which, like humans, express CETP is not known. The objective of this study was to determine the net effect of human apoC1 on atherosclerosis in rabbits, a species with naturally high CETP activity but dysfunctional apoC1.

GENERATION OF TRANSGENIC RABBITS EXPRESSING HUMAN APOC1

Construct

Gene expression pattern

Plasma distribution of human ApoC1

One transgenic line harboring 3 copies of the transgene was successfully established. The EF1 α promoter led to ubiquitous expression of the transgene, with the highest levels in heart and pancreas. In plasma, most human apoC1 co-localized with HDL, with smaller amounts present in the low-density lipoprotein (LDL) as well as in the lipoprotein-free fraction

CHARACTERISTICS OF CHOW-FED RABBITS

	WT	HuApoC1Tg
Body weight (g)	2107 $\pm$ 189	2111 $\pm$ 151
Total cholesterol	1.05 $\pm$ 0.17	1.18 $\pm$ 0.32
Free cholesterol	0.05 $\pm$ 0.03	0.07 $\pm$ 0.05
Cholesteryl esters	1.01 $\pm$ 0.15	1.11 $\pm$ 0.28
Triglycerides	0.73 $\pm$ 0.24	0.89 $\pm$ 0.32
Phospholipids	0.81 $\pm$ 0.11	0.83 $\pm$ 0.18
Free fatty acids	0.50 $\pm$ 0.17	0.53 $\pm$ 0.27
HDL-cholesterol	0.73 $\pm$ 0.11	0.83 $\pm$ 0.20 *
Non-HDL-C	0.32 $\pm$ 0.10	0.30 $\pm$ 0.08
LpB- to HDL-C	0.43 $\pm$ 0.09	0.36 $\pm$ 0.02 *
TRL- to HDL-C	0.14 $\pm$ 0.09	0.07 $\pm$ 0.02 *
Human ApoC1	N.D.	4.59 $\pm$ 2.37

HuApoC1Tg rabbits had higher HDL-C and lower LpB to HDL ratio than WT rabbits. This protective lipid profile might relate to lower CETP activity in HuApoC1Tg rabbits. The decrease in specific CETP activity indicates that the expression of human ApoC1 successfully restores CETP inhibition in HuApoC1Tg rabbits.

Cholesterol profile

CETP activity and mass

	WT Chow n=14	HuApoC1Tg Chow n=11
CE transfer rate (AU/min)	516 $\pm$ 72	466 $\pm$ 53 *
CETP mass (mg/l)	1.19 $\pm$ 0.13	1.30 $\pm$ 0.39
Specific activity (AU/min/ng)	43.7 $\pm$ 6.1	37.5 $\pm$ 7.5 *

CHARACTERISTICS OF RABBITS ON A HC DIET

	WT	HuApoC1Tg
Body weight (g)	3557 $\pm$ 508	3327 $\pm$ 254
Total cholesterol	32.35 $\pm$ 5.14	31.76 $\pm$ 6.98
Free cholesterol	4.28 $\pm$ 0.65	3.93 $\pm$ 0.54
Cholesteryl esters	27.62 $\pm$ 5.40	25.84 $\pm$ 6.59
Triglycerides	0.96 $\pm$ 0.38	0.98 $\pm$ 0.53
Phospholipids	6.86 $\pm$ 1.91	6.65 $\pm$ 1.27
Free fatty acids	1.35 $\pm$ 0.42	1.17 $\pm$ 0.36
HDL-cholesterol	0.83 $\pm$ 0.34	1.08 $\pm$ 0.51 *
Non-HDL-C	30.53 $\pm$ 6.33	30.43 $\pm$ 7.19
LpB- to HDL-C	39.35 $\pm$ 12.97	31.95 $\pm$ 13.78 *
TRL- to HDL-C	27.63 $\pm$ 9.11	21.98 $\pm$ 9.48 *
ApoA1	619.4 $\pm$ 233.8	648.3 $\pm$ 208.6
ApoAB	6645 $\pm$ 1359	6813 $\pm$ 1700
Human ApoC1	N.D.	62.02 $\pm$ 19.71

Cholesterol profile

CETP activity

After 8 weeks of a hypercholesterolemic diet (HC diet), HuApoC1Tg rabbits still had higher HDL cholesterol levels than WT rabbits. HDL isolated from HuApoC1Tg rabbits showed lower ability to interact with CETP. This translates into more protective lipid profile as reflected by the lower LpB to HDL ratio in HuApoC1Tg rabbits.

HDL PARAMETERS IN RABBITS ON A HC DIET

HDL size

Protective roles of HDL

HuApoC1Tg rabbits displayed higher amounts of large-sized HDL. PON-1 activity was significantly increased in HuApoC1Tg rabbits, together with a significant 16% decrease in the plasma levels of lipid oxidation marker 12(S)-HODE (71.1 $\pm$ 4.4 *versus* 84.0 $\pm$ 10.0 in HuApoC1Tg and WT rabbits, respectively, $p < 0.05$).

ATHEROSCLEROSIS IN RABBITS ON A HC DIET

After 8 week of HC diet, aortas from HuApoC1Tg rabbits showed a 21.9% reduction in atherosclerotic lesions as compared with WT rabbits. HE staining (A, B), RAM11 detection (C, D) and Masson Trichrome staining (E, F) of aortic lesions showed similar thickening of arterial intima and enrichment in macrophages and collagen fibers in both groups.

CONCLUSION

Expression of fully functional human apoC1 in transgenic rabbit plasma inhibits CETP activity, increases plasma HDL levels and functionality and consequently reduces the extent of atherosclerosis. ApoC1, as a natural and endogenous CETP inhibitor, arises as an alternative to pharmacological CETP inhibitors, which are still challenged in clinical studies. It emerges as a novel candidate to raise HDL levels and to fight against atherosclerosis in humans.