Effect of Human Apolipoprotein A-I Gene on Vasoactive Properties of High Density Lipoproteins in Rats of Different Age

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 5, pp. 506-508, May, 1998
Original article submitted April 22, 1997

Vasodilatory activity of high density lipoproteins and the content of apolipoprotein A-I in these particles decreases in senescent rats in comparison with adult animals. Implantation of human apolipoprotein A-I gene increases the content of this apolipoprotein in high density lipoproteins and improves their vasodilatory effect.

Key Words: *gene; lipoproteins; vessels; aging*

Changes in the content and proportion between various circulating lipoprotein complexes (dyslipoproteinemias) can modulate metabolic and functional alterations in the cardiovascular system during aging and atherosclerosis development [1,5]. Lipoprotein (LP) classes differ in their acceptor and transporting functions, while functional properties of different LP depend on their apoprotein constituents [7]. In particular, antiatherogenic properties of high density lipoproteins (HDL) are determined by the presence of apolipoprotein (apo) A-I.

LP complexes act as effective regulators of vascular reactivity [11]. We previously demonstrated that vasoactive effects of LP decrease during aging [4]. It was shown that implantation of human apo-A-I gene to rats modulates plasma content of different LP classes in senescent animals [3].

In light of this it was interesting to study age-related differences in the effect of implanted human apo-A-I gene on vasodilatory properties and apoprotein spectrum of HDL.

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MATERIALS AND METHODS

Experiments were carried out on adult (6-8-month-old) and senescent (26-28-month-old) Wistar rats. Apo-A-I gene was cloned from the library of human genes in the λ Charon 4A vector. Screening of recombinant phages, selection of molecular construction, isolation of plasmid DNA, lysosome preparation and expression of transplanted gene were previously described in detail [2]. The gene was injected into the liver in a liposome suspension (100 μl/100 g body weight). One milliliter of liposome suspension contained 13 mg lipids and 400 μg plasmid DNA, of which 7-8% was incorporated into liposomes.

All experiments were performed 48 h after gene implantation.

Different classes of LP were isolated as described previously [8] and protein composition of HDL was determined [10]. Contractile activity of isolated vascular segment from rat thoracic aorta was evaluated as described elsewhere [9] with some modifications.

0007-4888/98/0005-0447$20.00

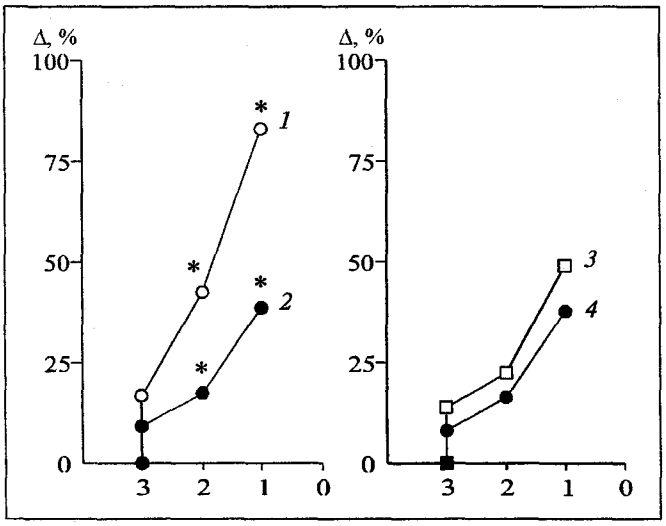

Fig. 1. Relaxation of rat thoracic aorta segment from rats of different ages induced by high density lipoproteins (HDL) isolated from adult and senescent rats. Abscissa: logarithm of HDL concentration, mg protein/ml fraction; ordinate: relaxation of vascular segment, % of initial contraction induced by norepinephrine (100 nM). *1*) vessel segment and HDL from adult rats; *2*) vessel segment and HDL from senescent rats; *3*) vessel segment from adult rats+HDL from senescent rats; *4*) vessel segment from senescent rats+HDL from adult rats.

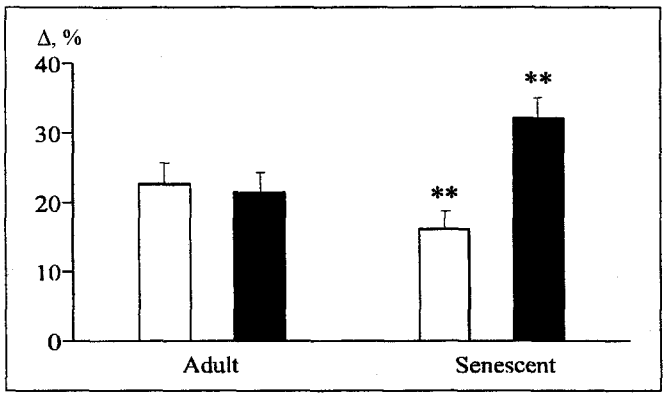

Fig. 2. Effect of implantation of human apolipoprotein A-I gene to rats of different age on relaxation of norepinephrine (100 nM)-precontracted isolated segment of the thoracic aorta from age-matched rats in the presence of high-density lipoproteins (100 nM). Light bars: control animals; shaded bars: rats with implanted human apolipoprotein A-I gene.

The data were processed statistically using the Student t test (95% significance).

RESULTS

In senescent rats vasodilatory effect of HDL was less pronounced than in adult animals (Fig. 1, *1*, *2*). These shifts can be due to age-related changes in both vascular reactivity and HDL properties. In the next experimental series, a blood vessel segment from senescent animal was incubated with LP from adult rat and vise-versa. Marked age-related shifts were observed. For instance, HDL from senescent rats induced less pronounced relaxation of vessel segment from adult rats than HDL from adult rats. Similarly, HDL from adult rats incubated with vessel segment from senescent animals elicited a weaker vasodilatory effect (Fig. 1, *3*, *4*). Thus, vasodilation was less pronounced in all experiments with LP or/and vessel segments from senescent rats. Consequently, not only vascular sensitivity to HDL but also their vasodilatory effect decreases during aging. The latter can be due to age-related alterations in protein and lipid composition of HDL.

Taking into account that human apo-A-I gene considerably changes the proportion between different plasma LP in senescent rats, in the next experimental series we explored the effect of this gene on vascular effects of HDL. It was found that HDL for senescent rats with implanted human apo-A-I gene exerts a more pronounced vasodilatory effect on the vascular segment from senescent rats than HDL from senescent controls. Implantation of human apo-A-I gene to adult rats had no effect on the vasodilatory properties of HDL (Fig. 2). In senescent rats with implanted human apo-A-I gene, vasodilatory effect of HDL not only returned to normal but also surpassed the effect of HDL from adult rats. This suggests that implantation of human apo-A-I gene to senescent rats improves vasodilatory effects

Table 1. Relative Content of Apolipoproteins in Plasma HDL from Rats f Different Age after Implantation of Human Apo-A-I gene, % (*M±m*)

Fraction	Molecular weight, kD	Adult		Senescent	
		control (*n*=30)	experiment (*n*=48)	control (*n*=30)	experiment (*n*=48)
Apo-C	12.5	<1	<1	<1	3.7±0.2
Apo-A-I	26	57.2±3.0	59.8±4.5	27.0±2.2**	36.6±2.4*
Apo-E	35	17.6±1.0	18.2±1.2	22.1±1.4**	29.7±2.1
Apo-A-IV	51	12.3±0.6	10.3±0.2*	21.7±2.2**	16.5±1.2*
Apo-A-I (2)	67	12.5±1.6	12.2±1.9	25.1±2.7**	17.2±1.2*
High-molecular-weight proteins	80	<1	<1	3.5±0.2	<1

Note. $p<0.05$: *compared with the control, ** compared with adult animals.

of HDL despite age-related shifts in vascular reactivity.

It can be hypothesized that these changes result from modifications of the protein composition of HDL particles. Implantation of human apo-A-I gene increases plasma HDL concentration by 30% in senescent animals (0.672±0.032 *vs.* 0.517±0.25 mg protein/ml plasma) and by 20% in adult rats (0.616± 0.024 vs.0.512±0.040 mg protein/ml plasma) and alters HDL apolipoprotein spectrum. As seen from Table 1, plasma content of apo-A-I decreases with age. Implantation of the apo-A-I gene has no effect on this parameter in adult rats, while in senescent animals it increases the relative content of apo-A-I by 36% but does not recover it to normal.

Thus, implantation of human apo-A-I gene to senescent rats increases plasma HDL concentration and the content of apo-A-I in HDL, enhancing their vasodilatory potential. Changes in lipid composition of HDL particles can contribute to these functional shifts. It has been shown that implantation of human apo-A-I gene to senescent animals increases the content of phospholipids, induces redistribution of cholesterol fractions, and improves acceptor and transport properties of HDL particles [6].

Our findings suggest that the synthesis of apo-A-I changes the proportion between different LP classes and induces changes in functional properties of HDL particles.

REFERENCES

1. A. N. Klinov and N. G. Nikul'cheva, *Lipoproteins, Dyslipoproteinemias, and Atherosclerosis* [in Russian], Leningrad (1984).
2. V. V. Frol'kis, V. A. Kordyum, L. N. Bogatskaya, *et al., Biopolimery i Kletki*, **7**, No. 2, 103-109 (1991).
3. V. V. Frol'kis, V. A. Kordyum, S. N. Novikova, *et al., Dokl. Akad. Nauk SSSR*, **316**, 1264-1267 (1991).
4. V. V. Frol'kis, O. K. Kul'chitskii, N. V. Sykalo, *et al., Dokl. Akad. Nauk Ukrainy*, No. 5, 132-136 (1995).
5. V. V. Frol'kis, V. V. Bezrukov, and O. K. Kul'chitskii, *The Aging Cardiovascular System. Physiology and Pathology*. New York (1996).
6. V. V. Frol'kis, V. A. Kordyum, L. N. Bogatskaya, *et al., Arch. Gerontol. Geriatr*, **13**, 225-236 (1991).
7. A. M. Gotto and H. J. Pownall, *Manual of Lipid Disorders*. Baltimore (1992).
8. F. T. Lindgren, L. C. Jencen, and F. T. Hatch, in: *Blood Lipids and Lipoproteins: Quantification, Composition, and Metabolism*, Chaper Inc. (1972), pp. 181-274.
9. L. Sjolin, F. Hellstrand, and B. Clementz, *Acta Physiol. Scand.*, **102**, 32A-33A (1978).
10. I. B. Swaney and K. S. Kuehl., *Biochim. Biophys. Acta*, **446**, 561-565 (1976).
11. Yu. P. Vedernikov, V. Z. Lankin, A. L. Tikhadze, and A. M. Vikhert, *Basic Res. Cardiol.*, **83**, 590-596 (1988).