ANTIOXIDANT ACTIVITY OF HIGH DENSITY LIPOPROTEINS IN VIVO

A. N. Klimov*, V. S. Gurevich, A. A. Nikiforova, L. V. Shatilina, A. A. Kuz'min, S. L. Plavinskii, and N. P. Teryukova

UDC 615.272.014.425:547.963.915].015.4.07

KEY WORDS: high-density lipoproteins; low-density lipoproteins; lipid peroxidation

Low-density lipoproteins (LDL), when isolated from blood plasma, are readily oxidized during keeping in aqueous solutions [4, 5], and also on incubation with endothelial and smooth-muscle cells from the aorta [8, 17]. It has been shown that when LDL have been oxidized, on interacting with macrophages they are ingested much more rapidly by these cells than native LDL [8, 12]. This ingestion, which is effected through the participation of scavenger receptors, leads to accumulation of cholesterol esters in the macrophages and to transformation of the macrophagal cell into a foam cell [8, 17]. It has been suggested that oxidation of LDL (more accurately, peroxidation of the lipids in LDL) increases their atherogeneity and plays an important role in the pathogenesis of atherosclerosis [16]. There is evidence that lipoproteins of another class, namely high-density lipoproteins (HDL), can delay peroxidation of LDL in vitro, whether spontaneous [1] or induced by iron or copper ions [2, 11, 13], by xanthine–xanthine oxidase [1], and by endothelial cells [13]. There is also evidence that the antioxidant effect is a characteristic feature mainly of the HDL_3 subclass [2]. The question arises whether the protective action of HDL is manifested against peroxidation of LDL only in experiments in vitro, or whether it is also exhibited in the intact animal, in which, as we know, there are other powerful antioxidant systems. A positive answer to the second part of the question would mean the establishment of yet another important property of HDL, namely its ability to protect LDL against peroxidation. This property of HDL, together with their participation in "reverse" cholesterol transport, would undoubtedly broaden our ideas on this class of lipoproteins, deservedly called "antiatherogenic."

The aim of this investigation was to study the effect of intravenous injection of a large dose of human HDL_3 into rabbits with experimental hypercholesterolemia on the content of primary lipid peroxidation products in the rabbit blood, and also to determine correlation between the HDL level and the content of lipid peroxidation products in the blood plasma of healthy individuals and patients with ischemic heart disease (IHD).

EXPERIMENTAL METHOD

To study the protective effect of HDL in experiments in vivo male rabbits weighing 2.0-2.5 kg were used. The animals were kept on a vegetable diet with the addition of 0.5% cholesterol for 3-4 weeks in order to induce moderate hypercholesterolemia in the animals, accompanied by an increase in the content of lipid hydroperoxides in LDL [3]. The subclass of high-density lipoproteins (HDL_3) was isolated from healthy human blood by the usual method, by successive ultracentrifugation in NABR solution with a density of 1.125-1.210 g/ml with the addition of EDTA in a concentration of 1 mg/ml. The isolated HDL_3 were dialyzed against 0.9% NaCl solution without EDTA for 24 h, after which it was injected intravenously into two rabbits with experimental hypercholesterolemia in a dose of 200 mg

*Academician of Russian Academy of Medical Sciences.

Department of Biochemistry, Institute of Experimental Medicine, Russian Academy of Medical Sciences, and Research Institute of Cardiology, Ministry of Health of Russia, St. Petersburg. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 7, pp. 40-42, July, 1992. Original article submitted December 20, 1991.

 0007-4888/92/0007-0960$12.50

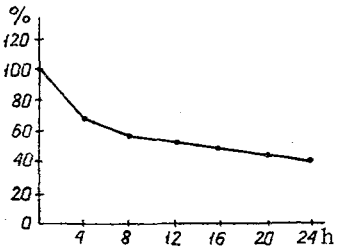

Fig. 1

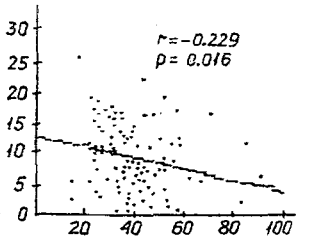

Fig. 2

Fig. 1. Elimination of HDL, estimated as Chs, from blood plasma of hypercholesterolemic rabbits after intravenous injection of human HDL_3. Abscissa, time after intravenous injection of HDL (in h); ordinate, Chs of HDL concentration after injection of HDL_3 into animals (in % of initial level observed after 5 min).

Fig. 2. Correlation between Chs of HDL level in blood plasma and concentration of conjugated dienes in combined group of healthy individuals and patients with IHD. Abscissa, Chs of HDL (in mg/dl); ordinate, conjugated dienes (in nanomoles/ml).

(as protein) per animal. Two other rabbits with experimental hypercholesterolemia (control) were given an intravenous injection of human albumin (200 mg), dissolved in 0.9% NaCl solution. Blood was taken from the animals before injection of the preparations and 6 and 24 h after injection. To discover correlation between levels of HDL and lipid peroxidation products, blood samples taken from 47 healthy blood donors (average age 35 years) and from 64 men with unstable angina (average age 46 years), under treatment at the clinic of the St. Petersburg Research Institute of Cardiology, were analyzed. The blood samples were taken after the subjects had been deprived of food for 12 h, into tubes containing EDTA. Concentrations of total cholesterol, triglycerides, and cholesterol of HDL were determined by the usual methods on a "Technicon AA-2" automatic analyzer. Cholesterol of LDL was calculated by Friedwald's formula [13]. Concentrations of conjugated dienes and trienes, reflecting accumulation of hydroperoxides in polyene lipids, were estimated in methanol–hexane (5:1 by volume) extracts of lipids, on the basis of measurement of the characteristic absorption for conjugated dienes at 233 nm [14], and for trienes at 277 nm [15]. The calculations were done on the basis of molar coefficients of extinction of $2.1 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ and $2.3 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ for conjugated dienes and trienes respectively, and expressed in nanomoles/ml plasma. The malonic dialdehyde concentration was determined by the method in [16] on the basis of the reaction with 2-thiobarbituric acid, and expressed in nanomoles/ml plasma, using a coefficient of molar extinction of $1.56 \cdot 10^5$ $M^{-1}cm^{-1}$. The content of Schiff bases was estimated by the method in [17], by measuring the intensity of fluorescence with a peak of absorption at 340-375 nm and a peak of emission at 420-490 nm. The concentration of Schiff bases was expressed in relative fluorescence units/ml plasma.

EXPERIMENTAL RESULTS

The preliminary experiments showed that after a single intravenous injection of human HDL_3 in a large dose there was a sharp rise of the level of these lipoproteins in the plasma. Whereas before injection of the lipoproteins the cholesterol of HDL level in the rabbits varied between 6 and 29 mg/dl 5 min after injection of HDL_3 the cholesterol level of the total HDL fraction reached 60-110 mg/dl. The level of these lipoproteins then fell gradually, and toward the end of the 1st day it was about 50% of that observed 5 min after injection of HDL_3 into the animals (Fig. 1). We accordingly were able to choose the dose of HDL_3 for intravenous injection and the time of taking blood in order to determine primary lipid peroxidation products.

As was pointed out in the section "Experimental Method," the experimental animals had hypercholesterolemia: the plasma cholesterol level before the rabbits received an injection of HDL_3 or albumin was about 700 mg/dl. As early as 6 h after intravenous injection of HDL_3 a statistically significant ($p < 0.01$) reduction of the serum concentrations of conjugated dienes and trienes was observed, and it continued (for conjugated dienes) for 24 h

TABLE 1. Blood Levels of Lipid Peroxidation Products in Hypercholesterolemic Rabbits after Intravenous Injection of Human HDL (n = 4) and Albumin (n = 3)

Preparation injected	Time of taking blood, h	Conjugated dienes, % (nanomoles/	Conjugated trienes, % (nanomoles/ml)
HDL_3	Time of taking	100,0 (115,8)	100,0 (45,8)
	6 h	67,0*	65,8*
	24 h	60,9*	83,2
Albumin	Before injection	100,0 (77,9)	100,0 (32,3)
	6 h	91,3	84,2
	24 h	96,8	77,5**

Legend. *$p < 0.01$, **$p < 0.05$.

TABLE 2. Correlation between Cholesterol of LDL and HDL Levels and Concentrations of Lipid Peroxidation (LPO) Products in Blood Plasma from Combined Group of Healthy Subjects (n = 47) and Patients with IHD (n = 64)

LPO products	Cholesterol		Cholesterol of HDL	
	r	*P*	*r*	*P*
Conjugated dienes	0,116	0,253	—0,236	0,014
Conjugated trienes	0,184	0,058	—0,076	0,438
Malonic dialdehyde	0,160	0,099	—0,018	0,855
Schiff bases	0,166	0,088	—0,091	0,353

(Table 1). In the control experiments, after intravenous injection of albumin into the hypercholesterolemic rabbits, the very small decrease in the concentrations of these conjugates was not statistically significant ($p > 0.05$).

In the second part of the investigation we studied correlation between the HDL level and concentrations of lipid peroxidation products in blood plasma from healthy individuals and patients with IHD.

The data in Table 2 and Fig. 2 show significant negative correlation between level of Chs of HDL and concentrations of conjugated dienes in human blood plasma. As regards the other lipid peroxidation products, the same type of correlation tendency (although, admittedly, not statistically significant), was observed between the HDL level and conjugated trienes and Schiff bases. This undoubtedly points to a general rule governing the phenomenon: the lower the blood HDL level, the higher its level of lipid peroxidation products. It follows from the data in Fig. 2 that if we go from very low concentrations of Chs of HDL (15 mg/dl) to very high (90 mg/dl) the average concentration of conjugated dienes fell from 11 mg/dl to 5 mg/dl, i.e., by a little more than twice. This suggests that the level of conjugated dienes, like that of other peroxidation parameters (see the coefficients of correlation in Table 2) can be significantly changed only as a result of sudden drops of the HDL concentration. It is possible to make the perfectly realistic assumption (which also follows from Fig. 2) that in individuals with hypoalpha-lipoproteinemia (Chs of HDL under 35 mg/dl) the concentration of lipid peroxidation products will be higher, whereas in individuals with hyperalpha-lipoproteinemia (Chs of HDL over 75 mg/dl) will be lower than in subjects with a normal level of this parameter.

On the whole the data showing negative correlation between the HDL level and the concentration of primary lipid peroxidation products in the blood plasma, combined with the discovery of the antioxidant effect of HDL in experiments on rabbits provide evidence of the universal character of the protective antiatherogenic action of this class of lipoproteins. The mechanism of this action of HDL is still far from clear. Different points of view, based on experiments in vitro [9, 11], have been expressed but they require further confirmation and clarification.

LITERATURE CITED

1. A. N. Klimov, P. A. Kozhemyakin, V. M. Pleskov, et al., Byull. Éksp. Biol. Med., No. 5, 550 (1987).
2. A. N. Klimov, A. A. Nikiforova, V. M. Pleskov, et al., Biokhimiya, **54**, No. 1, 118 (1989).
3. V. Z. Lankin, N. V. Kotelevtseva, A. K. Tikhaze, et al., Vopr. Med. Khim., No. 4, 513 (1976).
4. L. K. Bjornson, G. Gniewkowski, and H. J. Kayden, J. Lipid Res., **16**, 39 (1975).
5. A. Esterbauer, G. Jurgens, O. Quehenberger, et al., J. Lipid Res., **28**, 495 (1987).
6. B. Z. Fletcher, C. J. Dillared, and A. Y. Tappel, Analyt. Biochem., **52**, 497 (1973).
7. W. T. Friedwald, R. I. Levy, and D. S. Fredrickson, Clin. Chem., **18**, 499 (1972).
8. T. Henriksen, E. M. Mahoney, and D. Steinberg, Arteriosclerosis, **3**, 149 (1983).
9. V. W. H. van Hinsberg, M. Scheffer, L. Havekes, et al., Biochim. Biophys. Acta, **878**, 49 (1986).
10. M. I. Mackness, S. Arrol, and P. N. Durrington, FEBS Lett., **286**, No. 1/2, 152 (1991).
11. P. J. O'Brien, Can. J. Biochem., **47**, 485 (1969).
12. S. Parthasarathy, U. P. Steinbrecher, J. L. Witztum, et al., Proc. Nat. Acad. Sci. USA, **82**, 3000 (1985).
13. S. Parthasarathy, J. Barnett, and L. G. Fong, Biochim. Biophys. Acta, **1044**, 275 (1990).
14. F. S. Shenstone, Ultraviolet and Visible Spectroscopy of Lipids, New York (1971), pp. 77-91.
15. J. B. Smith, C. M. Ingerman, and M. J. Silver, J. Lab. Clin. Med., **88**, No. 1, 167 (1976).
16. D. Steinberg, Atheroscler. Rev., **18**, 1 (1988).
17. U. P. Steinbrecher, S. Parthasarathy, D. S. Leake, et al., Proc. Nat. Acad. Sci. USA, **81**, 3883 (1984).

ACTIVATION OF FREE-RADICAL REACTIONS AND CHANGES IN STATE OF THE ANTIOXIDATIVE PROTECTION SYSTEM IN THE BLOOD IN EXPERIMENTAL INFLUENZAL TOXICOINFECTION

N. V. Gorbunov, A. P. Volgarev, I. V. Brailovskaya, N. O. Bykova, N. F. Avrora, and O. I. Kiselev

UDC 616.98:578.832.1]-092.9-06:616-008.6]-07:616.153/.154-074

KEY WORDS: influenza; blood; free radicals; toxicosis

According to existing views acute virus infections are accompanied by profound changes of metabolism in the tissues [1, 2, 7], which can lead to activation of free-radical reactions [1], to accumulation of free-radical oxidation products ($O_2^{\cdot -}$, $HO^{\cdot}$, H_2O_2, nitric oxide, lipoperoxides, etc.) [1, 3], to a decrease in the content of natural antioxidants, to inhibition of enzymes of antioxidative protection, and to a change in the redox state and levels of hemoproteins and metallocomplexes (hemoglobin, cytochrome P-450, ceruloplasmin, transferrin, etc.), which may ultimately complicate the inflammatory processes and cause the development of hypoxia and toxicosis.

Influenza Research Institute, Ministry of Health of Russia. I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of Russia, St. Petersburg. (Presented by Academician of the Russian Academy of Medical Sciences S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 7, pp. 42-44, July, 1992. Original article submitted September 20, 1991.

0007-4888/92/0007-0963$12.50