AGE DIFFERENCES IN PLASMA LIPOPROTEIN COMPOSITION OF RATS WITH HEREDITARY RETINAL DEGENERATION

I. A. Ostapenko, V. G. Lozovskii, and
and E. Ya. Magracheva

UDC 617.735-003.8-056.7.07:[616.
153.96:577.112.856]-092.9

KEY WORDS: blood plasma lipoproteins; rat retina; hereditary degeneration

Retinitis pigmentosa (RP) is hereditary degeneration of the retina, manifested as loss of visual acuity and narrowing of the visual field, which ends in blindness [4]. Despite much research, the mechanism of this disease has not yet been explained. To study the causes of RP, various animals including Campbell rats, which suffer from hereditary degeneration of the retina [12], are used as models.

The results obtained are evidence that biochemical disturbances in RP take place not only in the retina and pigmented epithelium of the eye, but also in other organs and tissues. For instance, the uric acid concentration is raised in the blood of the affected patients, and similar changes are found in the blood of Campbell rats [3]. Disturbance of lipid peroxidation is found not only in the tissues of the eye (the retina and pigmented epithelium), but also in the brain tissues of rats with hereditary retinal degeneration [2]. Changes in the lipid composition and also disturbances of organic acid transport are found in the membranes of the proximal tubules of the kidneys in Campbell rats [1].

There is evidence in the literature on the presence of raised plasma levels of triglycerides (TG), cholesterol (ChS), and low-density lipoprotein cholesterol (LDL ChS) in patients with hereditary retinal degeneration compared with subjects of the control group [5]. These data indicate that a disturbance of lipid metabolism takes place in RP both in man and in animals.

The object of the investigation described below was accordingly compared blood plasma levels of lipids and lipoproteins (LP) in rats of two lines: Campbell (with hereditary retinal degeneration) and Wistar (control animals), of different ages, and also to study changes in the pattern of distribution of LP particles which may be associated with the development of hereditary retinal degeneration.

EXPERIMENTAL METHOD

Concentrations of TG, total ChS and LP Chs, and high-density lipoproteins (HDL) were determined on an AA-2 automatic analyzer ("Technicon") in 98 samples, each collected from the blood plasma of five or six Wistar or the same number of Campbell rats. Blood was taken from animals aged 15, 30, 45, 60, and 70-80 days after starvation for 12 h; eight to 12 samples were taken at each time.

Distributions of LP by flotation rate were obtained by an algorithm of numerical analysis [7], applied to the results of analytical ultracentrifugation of total LP fractions [10] of blood plasma from Wistar and Campbell rats, taken at the age of 30 and 45 days (12 specimens altogether).

High-speed flotation was carried out on a "Beckman Model E" ultracentrifuge at 26°C in analytical cells, with 12-mm two-sector inserts, and with a spinning speed of the rotor of 52,000 rpm. The conditions of flotation, for which the hydration density of the LP particles had to be below the relative density of the solvent, were obtained by adjusting the density of all the samples to d = 1.300 g/ml by dialysis for 24 h against 3.89 M NaBr, 0.196 M NaCl, and 0.01% EDTA.

Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 155-157, August, 1989. Original article submitted July 8, 1988.

 0007-4888/89/0008-1086 12.50

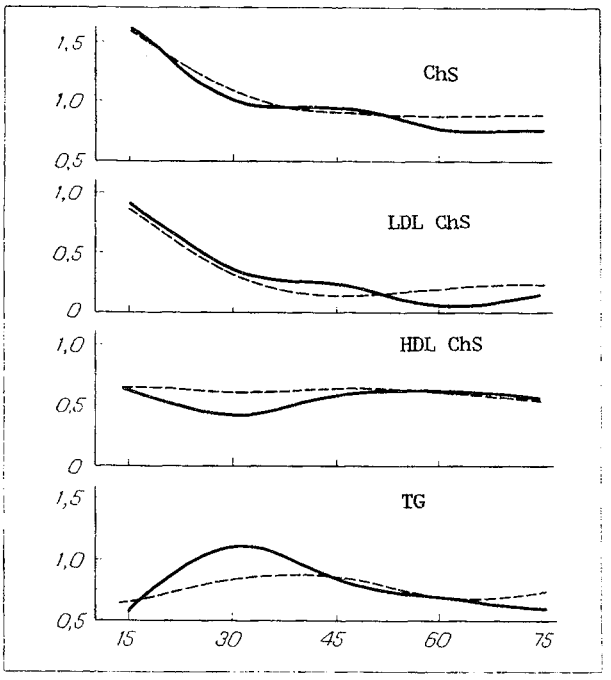

Fig. 1. Concentration of main classes of rat blood plasma LP at different times after birth. Abscissa, days after birth; ordinate, lipoprotein concentration (in g/liter).

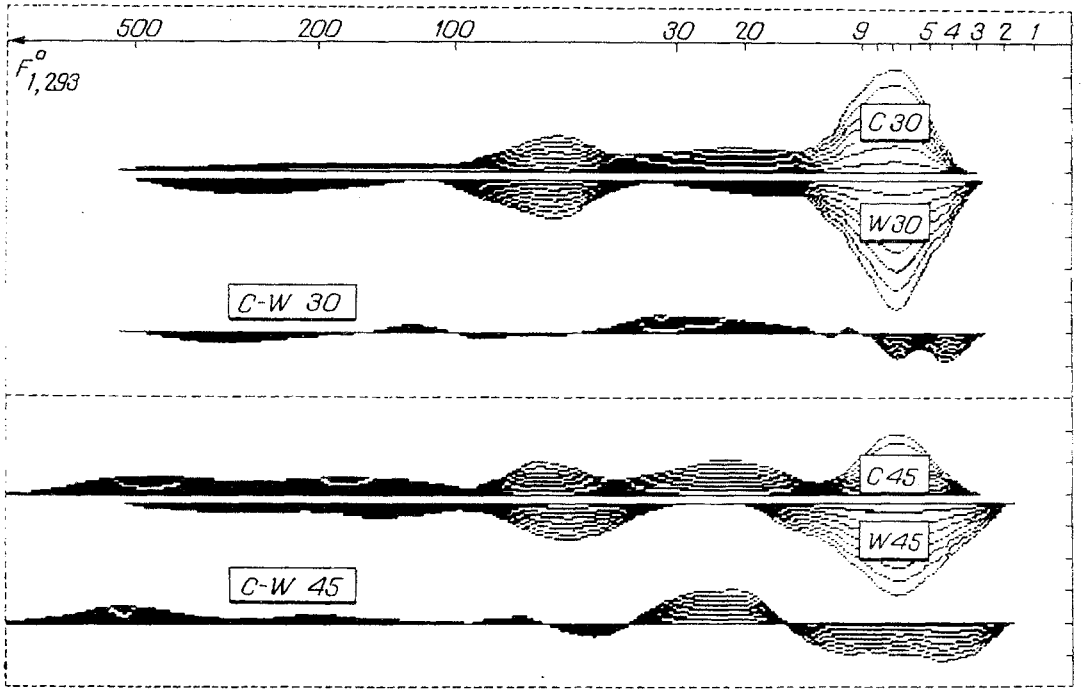

Fig. 2. Distribution of plasma LP of Wistar (W) and Campbell (C) rats by flotation rate on 30th and 45th days after birth.

In human blood plasma the concentration of LDL ChS is usually determined as the difference between the levels of total Chs and Chs of very low-density LP (VLDL ChS) and HDL ChS. It is assumed, when this is done, that the VLDL ChS level is one-fifth of the TG concentration in human blood plasma [8] and that by far the greater part of the

TG is contained in VLDL. This last assumption is true for all cases except hypertriglyceridemic human blood plasma. However, since the HDL concentration in rat blood plasma is 70% of the total circulating LP and since rat HDL differ from human HDL in their higher TG level, to determine the plasma LDL ChS level of rats an appropriate correction of the VLDL ChS concentration is needed.

The LDL ChS level was calculated by the equation LDL ChS = ChS — HDL ChS — (TG — HDL ChS/4) /5, reflecting species-dependent differences in LP metabolism in rat blood plasma, where the values of the TG, ChS, and HDL ChS concentrations were obtained experimentally.

Correlation analysis was carried out of concentrations of ChS and of the main classes of LP in the blood plasma of both strains of rats.

EXPERIMENTAL RESULTS

The concentrations of the main LP classes at different stages of development of rats of the two strains are given in Fig. 1. Continuous distributions were obtained by cubic interpolation of mean values of the experimental estimates for all times. The total ChS concentration in the blood plasma of both strains of rats fell steadily from 1.60 ± 0.04 to 0.80 ± 0.05 g/liter for the period from the 15th to the 60th days of life, due mainly to a corresponding fall in the LDL ChS concentration from 0.90 ± 0.05 to 0.20 ± 0.04 g/liter. The significance of the difference in the mean values of concentrations of total ChS and LDL ChS was found only when different ages were compared, not during comparison of different strains of rats. Concentrations of HDL ChS and TG in the blood plasma of Campbell rats showed no significant changes with age, and amounted to 0.75 ± 0.11 and 0.60 ± 0.05 g/liter respectively, whereas for Wistar rats a significant increase in the TG level was observed up to 1.00 ± 0.06 g/liter and a decrease in the concentration of HDL ChS to 0.4 ± 0.05 g/liter on the 30th day of life, leading to a marked difference at this time ($p < 0.01$) in the plasma HDL ChS levels of the Wistar and Campbell strains of rats. On the 15th and 30th days of life, the plasma ChS level in the rats of both strains had positive coefficients of correlation with concentrations of LDL and HDL but negative coefficients with VLDL. This dependence of the parameters, and also the fact that LDL and HDL correlate positively with each other but negatively with VLDL, indicates that LDL and HDL make equal contributions to the total ChS pool in the blood plasma of rats of both strains in the early stages of life. The small difference in the levels of significance of the coefficients of correlation of the matrices compared points to more active LP metabolism in Wistar than in Campbell rats in the period from the 15th to the 30th days after birth.

After the age of more than 30 days the parameters compared in rats of the two strains had highly significant negative correlation with one another.

Normalized distributions of the total plasma LP fraction of Wistar and Campbell rats on the 30th and 45th days after birth, based on flotation rates, are given in Fig. 2. Difference spectra illustrating quantitative relations between LP particles in mirror-image distributions revealed an extremely large number of "abnormal" LP particles in the blood plasma of Campbell rats whose flotation rate was slower than that of LDL but faster than that of HDL. The plasma HDL level in Wistar rats during the period from the 30th until the 45th days after birth rose from 1.3 ± 0.2 to 1.9 ± 0.3 g/liter, whereas in Campbell rats a fall of the HDL concentration from 1.6 ± 0.3 to 1.2 ± 0.2 g/liter and a rise of the level of "abnormal" LP particles from 0.7 ± 0.1 to 1.1 ± 0.2 g/liter, or up to 25% of the total plasma LP at each time, were observed. The flotation rate of the "abnormal" plasma HDL of Campbell rats was close in value to the flotation rate of "nascent" HDL, present in the blood of newborn infants [9], and also of persons with hereditary lecithin-cholesterol acyltransferase (LCAT) insufficiency. In children the presence of "nascent" HDL in the blood also is evidently connected with relative LCAT deficiency. "Nascent" HDL obtained during perfusion of the rat liver in the presence of LCAT inhibitor 5,5'-dithio-bis-nitrobenzoic acid differ from plasma HDL in chemical composition, shape, and structure of their LP particles. Electron-microscopy of negatively stained preparations of these particles give a distribution of disks from 4.5 nm in thickness and diameter to 20 nm, stacked in the form of rouleaux [11]. The flotation rate of aggregated discoid "nascent" HDL was somewhat higher than that of plasma HDL because of a decrease in the hydration density and coefficient of hydrodynamic action of LP particles with an increase in the degree of aggregation. The closely similar values of flotation rates of "nascent" and "abnormal" plasma HDL of 30-day-old Wistar and Campbell rats suggest that they are identical, although the degree of aggregation of the latter is a little higher.

Species differences in LP metabolism in rats compared with man include a high HDL concentration (70% of the total circulating LP level) and an increased concentration of TG in them. HDL, responsible for direct ChS transport, play a fundamental role in ChS homeostasis [6], on which the properties of cell membranes depend. In Campbell rats a marked disturbance of LP metabolism was observed: in month-old animals up to 25% of all plasma LP, but on the 45th day of life up to 50% of HDL remain in the form of "nascent" particles, and because of their structural defectiveness, they cannot perform the functions of normal HDL. This state of affairs must be reflected in all organ structures connected with ChS homeostasis.

Despite the fact that mean values of total ChS and LDL ChS of Wistar and Campbell rats were identical at all times of observation, the results obtained by analytical ultracentrifugation suggest that the development of RP in Campbell rats proceeds against the background of a hereditary disturbance of plasma LP metabolism.

Thus the blood plasma of Campbell rats at the age of 30 days shows a significant increase, compared with Wistar rats, in the concentration of HDL ChS. The presence of "abnormal" LP particles, occupying an intermediate position between LDL and HDL in their flotation rate and closely similar to "nascent" LP in their hydrodynamic properties, was found in the blood plasma of Campbell rats aged 30-45 days.

It can be postulated on the basis of these results that the development of RP in rats takes place against the background of a disturbance of the plasma LP spectrum.

LITERATURE CITED

1. V. M. Bresler, I. A. Ostapenko, E. N. Rebane, and G. G. Chusova, Byull. Éksp. Biol. Med., No. 11, 14 (1983).
2. M. G. Efimova, I. A. Ostapenko, and R. N. Étingof, Neirokhimiya, 6, No. 3, 406 (1987).
3. G. G. Chusova, I. A. Ostapenko, M. E. Shabanova, and R. F. Eliseeva, Byull. Éksp. Biol. Med., No. 11, 21 (1982).
4. G. J. Chader, G. Aquirre, S. Sanyal, et al., Heredity and Visual Development, ed. by J. B. Sheffield and S. R. Hilfer, Berlin (1985), pp. 1-30.
5. C. A. Converse, T. McLachlan, H. M. Hammer, et al., Retinal Degeneration: Experimental and Clinical Studies, New York (1985), pp. 63-74.
6. C. A. Drevon, A. D. Atlie, H. Pagburn, and D. Steinberg, J. Lipid Res., 22, 37 (1981).
7. A. M. Ewing, N. K. Freeman, and F. T. Lindgren, Adv. Lipid Res., 3, 25 (1965).
8. W. T. Friedwald, R. I. Levy, and D. S. Fredrickson, Clin. Chem., 18, 499 (1972).
9. R. L. Hamilton, L. S. Guo, T. E. Felker, et al., J. Lipid Res., 27, 967 (1986).
10. F. T. Lindgren, Analysis of Lipids and Lipoproteins, ed. by E. G. Perkins, Champaign, IL, (1975), pp. 204-224.
11. G. Uterman, H. J. Menzel, and K. H. Langer, FEBS Lett., 45, 29 (1974).
12. C. M. Yates, A. J. Dewar, H. Wison, et al., Exp. Eye Res., 18, 119 (1974).