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Abstract

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— BRIEF NOTE —

EFFECT OF NICOMOL ON HIGH DENSITY LIPOPROTEIN (HDL) SUBFRACTIONS, HDL_2^e AND HDL_3^e, SEPARATED BY ELECTROPHORESIS

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Abstract. High density lipoprotein (HDL) fractions were separated by dextran sulfate-Mg^{++} from sera of 5 healthy males before and after oral administration of a daily 1200 mg dose of Nicomol (2,2,6,6-tetrakis (nicotinoyloxymethyl) cyclohexanol; Cholexamin, Kyorin Pharmaceutical Co. Ltd.) for 2 weeks. The HDL fractions were further subdivided into HDL_2^e and HDL_3^e by Utermann's electrophoretic method on polyacrylamide gel containing lauric acid. These subfractions roughly corresponded to HDL_2 and HDL_3, respectively, which were separated by ultracentrifugation. Nicomol treatment increased the HDL_2^e/HDL_3^e ratio as is observed for the HDL_2/HDL_3 ratio, in addition to the increase in total HDL cholesterol concentration. The electrophoretic determination of HDL_2^e/HDL_3^e ratio in combination with HDL cholesterol level seemed to be clinically important in assessment of the alteration of HDL_2 and HDL_3 under physiological and pathological conditions.

Key words : HDL_2, HDL_3, HDL cholesterol, electrophoresis, Nicomol, nicotinic acid derivative.

A number of studies on high density lipoprotein and its subfractions, HDL_2 and HDL_3, have been made in recent years since the importance of HDL as a negative risk factor for atherosclerosis was re-evaluated. A generally accepted view is that HDL_2 is more important than HDL_3 in prevention of atherosclerosis (1, 2). However, the clinical application of HDL_2 and HDL_3 determination is still limited because of the time-consuming processes involved in the ultracentrifugal separation of HDL subfractions. In order to circumvent this problem, the authors employed the electrophoretic method described by Utermann (3) in subfractionation of HDL. Since nicotinic acid (4) and its derivative, Nicomol*** (5), are known to increase the HDL_2 fraction relative to the HDL_3, both separated by ultracentrifugation, the effect of Nicomol on the HDL subfractions separated by the electrophoretic method was studied in order to prove the validity of the

*** 2,2,6,6-tetrakis (nicotinoyloxymethyl) cyclohexanol; Cholexamin, Kyorin Pharmaceutical Co. Ltd.

separation method in terms of yielding a preferential increase in HDL_2 to HDL_3. The results reported in this communication indicate that Nicomol lowers the total cholesterol concentration and raises the HDL cholesterol concentration as well as the HDL_2/HDL_3 ratio in sera of healthy volunteers.

Materials and methods. Blood samples were taken from five healthy fasted adult males before and after daily administration of 1200 mg of Nicomol in 3 equally divided doses for two weeks. Sera were separated and analyzed for total and HDL cholesterol concentrations by an enzymatic method with T-Choles (Enzymatic, International Reagent Corp., Kobe) in combination with the dextran sulfate (molecular weight, 500,000; Pharmacia Fine Chemicals AB, Sweden) and Mg^{++} precipitation method of Kostner (6). HDL and its subfractions were separated also by ultracentrifugation according to the method of Yasugi *et al.* (7). Electrophoretic separation of HDL subfractions was performed by the method described by Utermann (3); the polyacrylamide gel disc electrophoresis (PAGE) of Davis (8) being modified by adding lauric acid to the separating gel (monomer concentration, 7.5%) and by photopolymerization of the gel. Each 40μl of HDL fractions separated by the precipitation method was applied to the gel after staining with Sudan Black B by the method of Ressler *et al.* (9). The ratio of HDL_2 to HDL_3 was estimated by densitometry of the stained lipoprotein bands at a wave length of 600 nm and with a slit length and width of 5 mm and 0.5 mm, respectively.

Results. Nicomol lowered the mean serum concentration of total cholesterol by 6 mg/dl from 163 to 157 mg/dl, while it increased the concentration of mean HDL cholesterol by 5.1 mg/dl from 45.9 to 51.0 mg/dl (Table 1), although the

TABLE 1. EEFFECTS OF NICOMOL ON SERUM CONCENTRATIONS OF TOTAL AND HDL CHOLESTEROL AND HDL_2/HDL_3 RATIO

Case	Concentrations of cholesterol (mg/dl)				HDL_2^c / HDL_3^c	
	Total		HDL			
	before	after	before	after	before	after
1	176	180	55.9	70.7	0.71	0.97
2	192	180	47.5	54.4	0.14	0.44
3	144	148	35.4	40.1	0.08	0.16
4	163	148	40.1	36.8	0.14	0.23
5	137	129	50.8	53.0	0.28	0.39
mean±SD	163±20	157±20	45.9±7.4	51.0±12.0	0.27±0.23	0.44±0.29
	— NS —		— NS —		— P<0.05 —	

NS, No significant difference in Student's *t* Test.

differences were not statistically significant. In our previous results of a similar

study with a larger number of cases, the differences were statistically significant (10, 11).

PAGE with lauric acid of HDL and its subfractions separated by ultracentrifugation is shown in Fig. 1. HDL in the original HDL fraction was seen as two separate bands, the fast one corresponding to a major component of HDL_3 and the slow one to HDL_2 and a minor component of HDL_3. Thus, the fast band was conveniently designated as HDL_3^e and the slow one as HDL_2^e, "e" standing for the abbreviation of electrophoresis, in order to distinguish HDL's separated by electrophoresis from those obtained by ultracentrifugation. Several sub-bands were observed in each fraction, but they were grouped into HDL_2^e or HDL_3^e. The ratio (1.5) of HDL_2^e to HDL_3^e obtained by densitometry of the separated bands was close to that (1.3) calculated from the cholesterol contents in HDL_2 and HDL_3 separated by ultracentifugation. The distribution of HDL_2^e and HDL_3^e varied widely among the individuals studied as Utermann has already indicated (3). This is shown in Fig.2. In some cases, HDL_2^e was seen as a tailing band of HDL_3^e, although it was not difficult to distinguish between the two bands.

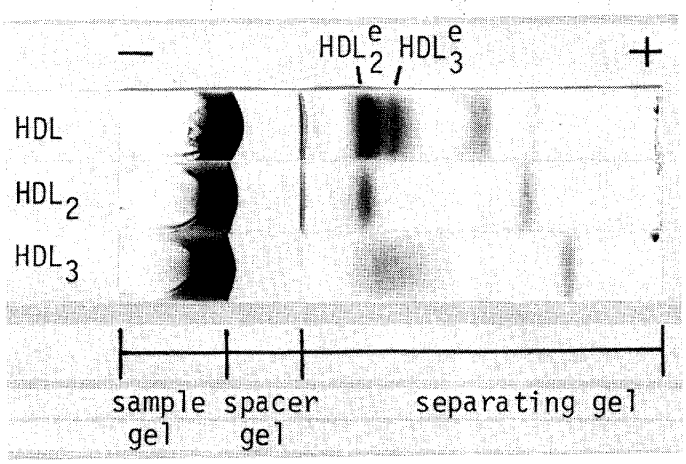

Fig. 1. PAGE with lauric acid of ultracentrifugally separated HDL, HDL_2 and HDL_3 fractions. Each 40μl of HDL and its subfractions, of which the volume was adjusted to the original one, was applied to the columns.

Although the ratio of HDL_2^e to HDL_3^e varied considerably among the cases studied, the relative increase in HDL_2^e to HDL_3^e by Nicomol was apparent by comparing the intensities of HDL_2^e and HDL_3^e before and after treatment (Fig. 2). In order to represent the difference numerically, the tracings of the electrophoregrams for Case 1 are presented in Fig.3. HDL_2^e/HDL_3^e ratios for the cases studied were obtained similarly and listed in Table 1. Although the ratios varied tremendously, ranging from 0.08 to 0.71 before treatment, they all increased after Nicomol treatment, and the difference was statistically significant.

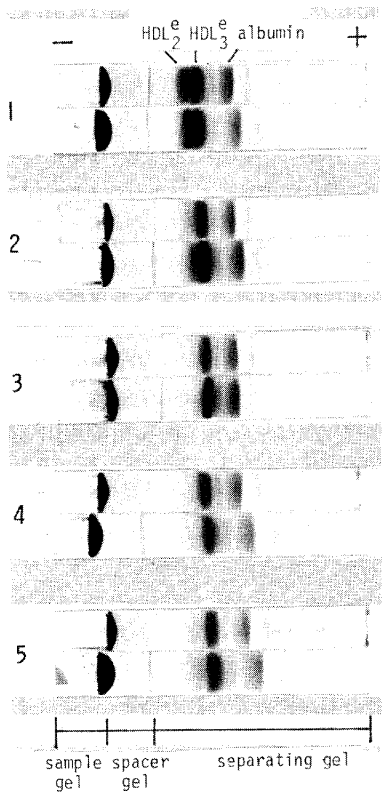

Fig. 2. PAGE with lauric acid of HDL fractions separated by the precipitation method before and after treatment with Nicomol. Pictures of upper row for each case are those before treatment and those of lower row after treatment. Each number represents a different case.

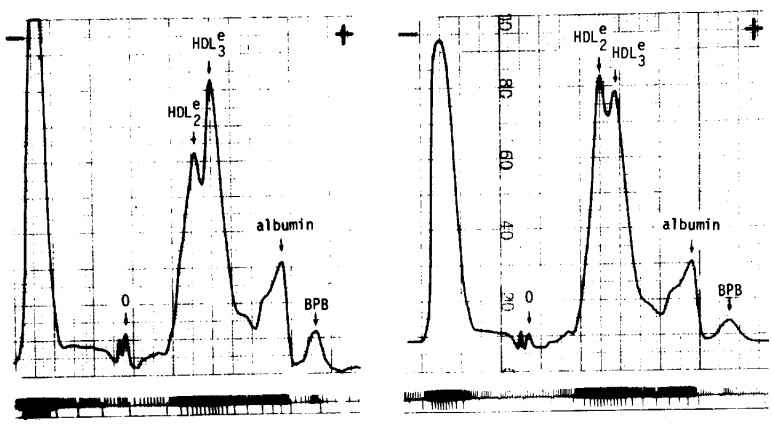

Fig. 3. Densitometric tracing of HDL bands separated by electrophoresis (Case 1). The left panel represents the electrophoretic pattern before Nicomol treatment and the right panel after Nicomol treatment. "0" denotes the origin, albumin the free and BPB the bound dye.

Discussion. The separation of HDL subfractions by electrophoresis has been studied by several workers. Narayan *et al.* (12) fractionated HDL by PAGE into several bands with their less clear correspondence to the density-dependent HDL subfractions. Since PAGE separates proteins according to their electric charge as well as molecular size, the poor correspondence may be explained by the fact that HDL_2 and HDL_3 can be further subdivided by isoelectric focusing (13, 14). Therefore, it seems reasonable to separate HDL_2 and HDL_3 by polyacrylamide gradient gel slab electrophoresis, which separates particles preferentially by their size. However, the resolution of HDL_2 and HDL_3 by this technique was not satisfactory either, because each HDL subclass contained HDL subcomponents overlapping each other with respect to their particle size (15). Utermann's (3) application of lauric acid to the original PAGE is a simple and convenient way of separating HDL_2 and HDL_3 as clearly discernible separate bands.

This method appears to separate HDL_2 and HDL_3 by the size of particle independent of the difference in the original particle charge probably due to addition of further negative charges from lauric acid to the lipoprotein particles. Faster mobilities of lipoprotein bands in the presence of lauric acid have been demonstrated by the authors (unpublished observation).

HDL_2 is still heterogeneous and has been further subdivided by Anderson *et al.* (16) into HDL_{2a} and HDL_{2b}. Although several minor subcomponents of HDL_2 were separated by Utermann's method, they tended to be rather continuous with respect to the electrophoretic mobility and could not be correlated to HDL_{2a} and HDL_{2b}. Slow-migrating HDL (17) has a density range (1.063-1.083) close to that of HDL_{2b} (1.063-1.100) (16), but its electrophoretic mobility was less than that of HDL_2^e (authors' unpublished observation). The physiological significance of HDL_2^e and HDL_3^e separated by PAGE with lauric acid is similar to that of HDL_2 and HDL_3, because both responded to the administration of Nicomol, giving rise to higher ratios of HDL_2/HDL_3 or HDL_2^e/HDL_3^e.

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