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Effect of Anticonvulsant Drugs on Plasma Total Cholesterol, High-Density Lipoprotein Cholesterol, and Apolipoproteins A and B in Children with Epilepsy (42189)

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Abstract. The effect of long-term anticonvulsant drug therapy with phenobarbital, phenytoin, carbamazepine, primidone, and valproic acid in epileptic children on plasma total cholesterol and high-density lipoprotein cholesterol (HDLC) was studied. Except valproic acid, all the drugs significantly increased the total cholesterol and HDLC, but the effect was more pronounced with HDLC. Among the subfractions of HDLC, almost all the increase due to drug therapy were in the HDLC-2 fraction. Treatment with antiepileptic drugs had no effect on HDLC-3. Apolipoprotein-A levels were significantly higher with drug therapy, but no effect was seen in the apolipoprotein-B levels. Plasma concentration of total cholesterol, HDLC, or its components was unaffected with valproic acid therapy.

Epidemiological studies from different parts of the world have demonstrated an independent, negative relationship between the plasma concentration of high density lipoprotein cholesterol (HDLC)[1] and the risk of coronary heart disease (CHD) (1, 2). Patients with CHD have a lower level of HDLC, and familial excess of HDLC has been associated with decreased risk.

Antiepileptic drugs such as phenobarbital, phenytoin, carbamazepine, primidone, and valproic acid have been used for the treatment of epilepsy (3). One of the beneficial side effects of these drugs in anticonvulsant therapy appears to be protection from CHD (4, 5). Administration of barbiturates to healthy volunteers has been shown to increase triglycerides, total cholesterol, low- and high-density lipoproteins (6, 7). Epileptic patients and normal subjects treated with phenytoin or carbamazepine either alone or in combination showed increased plasma HDLC (8–11). Similarly, anticonvulsant therapy with either single or a combination of several different drugs also resulted in a significant increase in the plasma HDLC levels (12, 13). Thus, if anticonvulsants have an effect in modulation of plasma HDLC levels, then use of these drugs may have a significant influence in the prevention of CHD.

All these above reports are concerned with the adult population, and no information is available on children. Further, in children, the effect of primidone or valproic acid therapy on HDLC is not known. The present study is therefore undertaken to see the effects of anticonvulsant treatment with phenobarbital, phenytoin, and carbamazepine, as well as primidone and valproic acid, as a single drug therapy on plasma levels of total cholesterol and HDLC in children. In addition, this study also deals with the effect of the above drugs on the subfractions of HDLC (HDLC-2 and HDLC-3), as well as apolipoproteins A and B.

Materials and Methods. Plasma samples from 82 epileptic children (42 male and 40 female) undergoing anticonvulsant drug therapy at the pediatric epilepsy clinic, Milwaukee Children's Hospital, Milwaukee, Wisconsin, were used in this study. All the children were on a single specific drug for at least 3 months, and the drug levels in plasma were being maintained at the optimal therapeutic concentration, (phenobarbital, 15–40 μg/ml; phenytoin, 10–20 μg/ml; carbamazepine, 8–12 μg/ml; primidone, 5–12 μg/ml; valproic acid, 50–100 μg/ml), under the supervision of the clinic's staff. The ages of the patients were between 6 and 18 years (mean ± SD = 9.5 ± 5.4), and all were in reasonably good health. Samples from 101 children (48 male and 53 female) of similar age (mean ± SD = 10.0

[1] Abbreviations used: HDLC, high-density lipoprotein cholesterol; HDLC-2 and HDLC-3, fractions 2 and 3 of high-density lipoprotein cholesterol; CHD, coronary heart disease; apo-A, apolipoprotein-A; apo-B, apolipoprotein-B.

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± 6.3) and sex who were on routine visits to the other clinics were used as controls. All the children were from the Milwaukee, Wisconsin, area and had normal triglyceride and cholesterol levels and no unusual dietary habits.

Blood samples were collected in 5-ml vacutainer tubes containing approximately 75 USP units of sodium heparin. Plasma was separated by centrifugation at 1500*g* for 10 min and stored at 4°C until used. All the work was completed within 48 hr after collection.

Total cholesterol was determined by an enzymatic method (14) on the Abbott-VP analyzer (Abbott Diagnostics, North Chicago, Ill.), using reagents from Abbott Diagnostics. The concentration of high-density lipoproteins was measured in the supernatants, obtained after precipitating the apolipoprotein-B-associated lipoproteins (very-low-density lipoproteins, low-density lipoproteins), using heparin–manganese chloride reagents as per the Lipid Research Clinics Program (15, 16). The HDLC levels were estimated by measuring the cholesterol content similar to the total cholesterol procedure.

The subfractions of the HDLC (HDLC-2 and HDLC-3) were separated by ultracentrifugation at a density of 1.125 g/ml using an air-driven ultracentrifuge (Airfuge, Beckman Instruments) (17). To 250 μl of the supernatant, obtained after heparin–$MnCl_2$ precipitation, 46 mg of finely ground dry potassium bromide was added. After mixing, 150 μl of the sample was transferred to the centrifuge tube and spun at 95,000 rpm using an air pressure of 30 psig (207 kPa, 149,000*g*) for 2.5 hr. At the end of centrifugation, the top 50 μl, containing the HDLC-2 fraction, and the bottom 50 μl, containing HDLC-3 fraction, were removed carefully and used for quantitation. The HDLC-2 and HDLC-3 fractions were quantitated by measuring their cholesterol content. Since HDLC and its subfractions were determined individually, the sum of these subfractions were not always equal to the total HDLC. In our experimental condition, the coefficient of variation of this technique was about 5–7%.

Levels of apolipoprotein-A and apolipoprotein-B were determined by the radial immunodiffusion procedure using reagent kits from the Calbiochem-Behring Corporation. Each sample was assayed in triplicate. Antibodies used in these kits were monospecific to the given antigen; and the precision, in our experimental conditions, was about 4–6% during a period of 6 months.

All the data are an average of two separate determinations and expressed as means ± standard deviation. The Student *t* test was employed for statistical analysis.

Results. *1. Effect on total cholesterol.* In epileptic children treated with phenobarbital, phenytoin, carbamazepine, or primidone, plasma total cholesterol increased significantly (Table I). Among these four drugs, carbamazepine had a greater effect than the others. In contrast to these four drugs, treatment with valproic acid did not show any change in the total cholesterol levels compared to the controls.

2. Effect on HDLC. Similar to the total cholesterol, patients on anticonvulsant drug therapy also had significantly higher levels of HDLC in their plasma (Table I). Compared to the controls, phenytoin-treated children had

TABLE I. EFFECT OF ANTICONVULSANT DRUG TREATMENT ON THE TOTAL CHOLESTEROL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDLC) IN PLASMA OF CHILDREN

		Total cholesterol		HDLC		Total Chol/HDLC ratio	
Treatment	Number of patients	mg/dl (mean ± SD)	*P* value vs control	mg/dl (mean ± SD)	*P* value vs control	mean ± SD	*P* value vs control
Controls (none)	101	156 ± 36	—	46 ± 13	—	3.52 ± 0.94	—
Phenobarbital	15	170 ± 29	<0.02	67 ± 15	<0.001	2.60 ± 0.81	<0.001
Phenytoin	15	165 ± 22	<0.02	57 ± 11	<0.001	2.72 ± 0.78	<0.001
Carbamazepine	22	175 ± 23	<0.01	62 ± 12	<0.001	2.63 ± 0.69	<0.001
Primidone	15	166 ± 25	<0.02	58 ± 13	<0.001	2.79 ± 0.77	<0.01
Valproic acid	15	159 ± 29	NS[a]	46 ± 10	NS	3.61 ± 0.88	NS

[a] NS = not significant.

TABLE II. EFFECT OF ANTICONVULSANT DRUG TREATMENT ON THE SUBFRACTIONS OF HIGH-DENSITY LIPOPROTEIN CHOLESTEROL (HDLC-2 and HDLC-3) IN PLASMA OF CHILDREN

Treatment	Number of patients	HDLC-2 Concentration mg/dl (mean ± SD)	HDLC-2 *P* value vs control	HDLC-3 Concentration mg/dl (mean ± SD)	HDLC-3 *P* value vs control
Controls (none)	101	32 ± 10	—	20 ± 5	—
Phenobarbital	15	48 ± 10	<0.001	22 ± 4	NS
Phenytoin	15	46 ± 9	<0.001	19 ± 3	NS
Carbamazepine	22	43 ± 11	<0.001	22 ± 4	NS
Primidone	15	44 ± 12	<0.001	20 ± 6	NS
Valproic acid	15	31 ± 12	NS[a]	21 ± 5	NS

[a] NS = not significant.

24% higher levels, whereas with the phenobarbital treatment the difference was 46%. HDLC levels did not increase in patients treated with valproic acid alone.

The calculated total cholesterol/HDLC ratio was lower significantly with the treatment of all the drugs, except valproic acid (Table I).

3. Effect on HDLC subfractions. Controls as well as patients have a higher amount of HDLC-2 than the HDLC-3 fraction (Table II). However, patients treated with either phenobarbital, phenytoin, carbamazepine, or primidone, had significantly higher HDLC-2 levels ($P < 0.001$). Valproic acid treatment had no effect on the HDLC-2 fraction. Unlike HDLC-2, the concentration of HDLC-3 did not change with the treatment of any of these drugs, and, in fact, it was almost equal in controls and in patients treated with the drugs.

4. Effect on apolipoproteins. Anticonvulsant therapy with phenobarbital, phenytoin, carbamazepine, and primidone had a significant positive effect on the plasma concentrations of apolipoprotein A (Table III). With the primidone-treated group it was 10% higher ($P < 0.01$) and in the carbamazepine-treated group it was 33% higher ($P < 0.001$) than the control group. Valproic acid therapy had no effect on the apo-A levels.

In contrast to apo-A, apo-B levels did not change significantly after treatment with any one of these drugs.

Discussion. This work with epileptic children clearly supports several earlier observations on adult patients that long-term treatment with antiepileptic drugs increases plasma cholesterol and HDLC levels.

Increased plasma cholesterol was observed when patients were treated with phenobarbital, phenytoin, or carbamazepine, either alone or

TABLE III. EFFECT OF ANTICONVULSANT DRUG THERAPY ON APOLIPOPROTEINS A AND B IN PLASMA OF CHILDREN

Treatment	Number of patients	Apolipoprotein-A Concentration, mg/dl (mean ± SD)	Apolipoprotein-A *P* value vs control	Apolipoprotein-B Concentration, mg/dl (mean ± SD)	Apolipoprotein-B *P* value vs control
Controls (none)	101	160 ± 21	—	72 ± 18	—
Phenobarbital	15	198 ± 25	<0.001	76 ± 11	NS
Phenytoin	15	209 ± 21	<0.001	71 ± 14	NS
Carbamazepine	22	212 ± 24	<0.001	68 ± 13	NS
Primidone	15	176 ± 20	<0.01	72 ± 16	NS
Valproic acid	15	156 ± 28	NS[a]	71 ± 20	NS

[a] NS = not significant.

in combination (8, 9, 11–13). However, the role of cholesterol in control of seizures is not clear. In experimentally induced seizures in rats, mice, and monkeys, a low serum cholesterol was associated with the increased seizures, whereas animals maintained on a high cholesterol diet were less susceptible to convulsive seizures (18, 19). In humans it was found that high cholesterol and high fat diets were beneficial in treatment of epilepsies of diverse etiologies (20–22). Further, a significant correlation was observed between the clinical improvement and rise in serum cholesterol during the course of treatment (21). However, in the present work no change in the total cholesterol levels was observed in patients undergoing valproic acid treatment. Therefore, increase in the serum total cholesterol may not be a requirement to control the seizures.

Similar to the total cholesterol, HDLC levels also were increased with the drug therapy. However, the rise was more pronounced with the HDLC. Further, except with valproic acid, the ratio of total cholesterol/HDLC decreased significantly by treatment with all the other four drugs. Therefore, even though both total cholesterol and HDLC rise with the therapy, the effect appears to be somewhat selective for HDLC.

Increased plasma HDLC levels have been observed with the anticonvulsant drugs (8–13), but none of these reports deal with its subfractions. Perhaps the most interesting observation in the present work is the effect on the HDLC subfractions. Wherever there is a rise in the HDLC, it is always in the HDLC-2 fraction, and no significant change is ever seen in the HDLC-3 with any one of these drugs. This probably shows that either the antiepileptic drugs have no effect on HDLC-3 or the HDLC-3 metabolism may be tightly regulated.

Recently, the roles of lipoprotein lipase and hepatic lipase have been implicated in the metabolism of HDLC and its subfractions. Taskinen and Nikkila (23) observed that the concentration of the HDLC-2 and its components, viz., cholesterol, phospholipid, and proteins, are all positively correlated with lipoprotein lipase activity. In rats treated with phenobarbital, activities of lipoprotein lipase and hepatic lipase have increased significantly (24). Perhaps in this regard it is reasonable to speculate that anticonvulsant drugs may have an effect on these two enzymes in children also.

Treatment with valproic acid did not affect the plasma total cholesterol, HDLC or its components. The lack of any positive change in total cholesterol and HDLC indicates that the changes in lipoproteins after anticonvulsive drug therapy appear to be drug related and not secondary to seizures per se.

Substantial evidence exists for the negative correlation between HDLC levels and coronary risk (1, 2). Although it is still not known whether the drug-induced elevation of HDLC alters the risk toward cardiovascular diseases, the present observations suggest that a long-term treatment with phenobarbital, phenytoin, carbamazepine, or primidone may be beneficial in decreasing this risk. In this regard, the earlier observations of Linden (4) and Livingston (5) are noteworthy because of low incidence of myocardial infarction in epileptic patients on long-term anticonvulsive drug therapy.

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1. Heiss G, Johnson NJ, Reiland S, Davis CE, Tyroler HA. The epidemiology of plasma high-density lipoprotein cholesterol levels. The lipid research clinics program prevalence study summary. Circulation **62:** IV116–IV136, 1980.
2. Gordon T, Castelli MP, Hjortland MC, Hannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. Amer J Med **62:** 707–714, 1977.
3. Reynolds EH. Drug treatment of epilepsy. Lancet **II:** 721–725, 1978.
4. Lindin V. Myocardial infarction in epileptics. Brit J Med **2:**87, 1975.
5. Livingston S. Phenytoin and serum cholesterol. Brit J Med **1:**386, 1976.
6. Durrington PN. Effect of phenobarbitone on plasma apolipoprotein B and plasma high density lipoprotein cholesterol in normal subjects. Clin Sci **56:**501–504, 1979.
7. Miller NE, Nestel PJ. Altered bile acid metabolism during treatment with phenobarbitone. Clin Sci Mol Med **45:**257–262, 1973.
8. Nikkila EA, Kaste M, Ehnholm C, Viikari J. Elevation of high density lipoprotein in epileptic patients treated with phenytoin. Acta Med Scand **204:**517–520, 1978.
9. Luoma PV, Myllylla VV, Sotaniemi EA, Hokkanen TEJ. Plasma HDL cholesterol and growth hormone

in epileptics treated with anticonvulsants. J Pharmacol Toxicol **47**:249–251, 1980.
10. Kaukola S, Manninen V, Neuvonen PJ, Malkonen M, Ehnholm C. Effect of phenytoin on serum lipoproteins in middle aged men. J Cardiovasc Pharmacol **3**:207–214, 1981.
11. Luoma PV, Myllyla VV, Sotaniemi EA, Hokkanen TEJ. Plasma HDL cholesterol in epileptics with elevated triglyceride and cholesterol. Acta Neurol Scand **60**:56–63, 1979.
12. Berlit P, Krause K-H, Heuck CC, Schellenberg B. Serum lipids and anticonvulsants. Acta Neurol Scand **66**:328–334, 1982.
13. O'Neill B, Callaghan N, Stapleton M, Molloy W. Serum elevation of high density lipoprotein (HDL) cholesterol in epileptic patients taking carbamazepine or phenytoin. Acta Neurol Scand **65**:104–109, 1982.
14. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem **20**:470–475, 1974.
15. Manual of Laboratory Operations. The Lipid Research Clinics Program. Volume I. Lipid and Lipoprotein Analysis. NHLBI, NIH, DHEW Publication No. (NIH) 75-628, 1974.
16. Warnick GR, Albers JJ. HDL cholesterol quantitation: Comparison of six precipitation methods and ultracentrifugation. In: Kappel L, ed. Report of the High Density Lipoprotein Methodology Workshop. USDHH, PHS, NIH Publication No. P2-1661, pp53–68, 1979.
17. Eyre J, Hammett F, Miller NE. A micro-method for the rapid ultracentrifugal separation of human plasma high density lipoprotein subfractions, HDL-2 and HDL-3. Clin Chim Acta **114**:225–231, 1981.
18. Alexander GJ, Kopeloff LM. Induced hypercholesterolemia and decreased susceptibility to seizures in experimental animals. Exp Neurol **32**:132–140, 1971.
19. Kopeloff LM, Alexander GJ. Serum cholesterol in monkeys with chronic epileptic foci. Life Sci **10**:869–876, 1971.
20. Aird RB, Gurchot C. Protective effect of cholesterol in experimental epilepsy. Arch Neurol Psychiatr **42**: 491–506, 1939.
21. Dekaban AS. Plasma lipids in epileptic children treated with the high fat diet. Arch Neurol **15**:177–184, 1966.
22. Millichap JG, Jones JD, Rudis BP. Mechanism of anticonvulsant action of ketogenic diet. Amer J Dis Child **107**:593–604, 1964.
23. Taskinen M-R, Nikkila EA. High density lipoprotein subfractions in relation to lipoprotein lipase activity in tissues in man. Evidence for reciprocal regulation of HDL-2 and HDL-3 levels by lipoprotein lipase. Clin Chim Acta **112**:325–332, 1981.
24. Goldberg DM, Wahid Roomi WM, Yu A. Modulation by phenobarbital of lipolytic activity in post-heparin plasma and tissues of the rat. Canad J Biochem **60**: 1077–1083, 1983.

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