Effect of phenobarbitone on plasma lipids in normal subjects

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Summary

1. Phenobarbitone in a dose of 180 mg daily was administered to ten normal subjects for 3 weeks. There was a significant increase in total plasma cholesterol, plasma low-density-lipoprotein cholesterol, plasma low-density-lipoprotein (LDL) triglycerides and plasma LDL protein. The increase in plasma LDL cholesterol accounted for the increase in total plasma cholesterol. There was a significant reduction in the ratio of LDL cholesterol to LDL protein.

2. No significant changes were observed in total plasma triglycerides, plasma very-low-density-lipoprotein (VLDL) triglycerides, plasma VLDL cholesterol or plasma VLDL protein.

3. Evidence that drug-metabolizing enzymes were induced by phenobarbitone was provided by an increase in antipyrine clearance. No relationship was observed between changes in plasma cholesterol and changes in antipyrine clearance. Serum y-glutamyl transpeptidase was also increased after phenobarbitone administration, the increase being unrelated to changes in antipyrine clearance or plasma cholesterol.

Key words: antipyrine clearance, cholesterol, y-glutamyl transpeptidase, low-density lipoproteins, microsomal enzyme induction, phenobarbitone, triglycerides, very-low-density lipoproteins.

Introduction

Phenobarbitone is known to induce hepatic microsomal enzymes (Conney, 1967), and it has recently been suggested that the induction of microsomal enzymes may be an important factor determining plasma lipid concentrations (Martin, Martin & Goldberg, 1975). The present study has examined the influence of phenobarbitone on plasma lipid concentrations and on the relation between these and antipyrine clearance, the latter being used as an index of hepatic microsomal enzyme activity.

Subjects and methods

Subjects and design of study

Seven healthy male and three healthy female volunteer subjects, aged 20–30 years, gave their informed consent to participate in the study, which had been approved by an ethical committee. All subjects maintained their normal diet throughout the investigation and avoided any excessive alcohol intake. None of the women was taking an oral contraceptive. All medication was excluded for at least 2 weeks before and during the study.

Each subject received phenobarbitone 180 mg daily (30 mg twice during the day and 120 mg at bed-time) orally for 3 weeks. The following were determined initially and at the end of the period on phenobarbitone: total plasma cholesterol and triglycerides, plasma very-low-density-lipoprotein (VLDL) cholesterol, plasma VLDL triglycerides, plasma VLDL protein, plasma low-density-lipoprotein cholesterol, plasma LDL triglycerides, plasma LDL protein, antipyrine clearance and serum y-glutamyl transpeptidase.

(1) Abbreviations: VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein.
γ-glutamyl transpeptidase activity. All blood samples for lipid analysis were collected without stasis after a fast for 11 h into bottles containing dipotassium EDTA and plasma was separated by centrifugation within 1 h of collection. Phenobarbitone concentrations were measured before the first dose of the day at the end of the second week.

Data from before and after phenobarbitone administration were compared by Student's paired t-test.

Methods

Ultracentrifugation of plasma. VLDL was obtained as the supernatant after ultracentrifugation of 5 ml of plasma on the day of collection for 24 h at 100,000 g (de Lalla & Gofman, 1954) (MSE Superspeed 50). The density of the infranatant was adjusted to 1.063 g/ml by addition of a solution of sodium chloride and potassium bromide (Havel, Howard & Bragdon, 1955) and LDL formed the supernatant after ultracentrifugation for a further 24 h.

Assay methods. Cholesterol and triglycerides in plasma and plasma density fractions were measured on the Technicon AutoAnalyzer, cholesterol by a modification of the Liebermann-Burchard reaction (Robertson & Cramp, 1970) and triglycerides by the standard semi-automated Technicon method (Kessler & Lederer, 1966).

Plasma VLDL and LDL protein concentrations were measured by a modification of the method of Lowry (Lowry, Rosebrough, Farr & Randall, 1951; Scanu & Page, 1959), human serum albumin (Sigma Chemical Co.) being used as the standard.

Serum γ-glutamyl transpeptidase was measured with the kit supplied by the Boehringer Corporation (Szasz, 1969).

Serum phenobarbitone concentrations were measured by gas–liquid chromatography (Toseland, Grove & Berry, 1972).

Antipyrine clearance. Antipyrine (1200 mg) was dissolved in 50 ml of water and administered orally to fasting subjects. Blood samples were taken at 10 min intervals for the first hour and then at 3, 5, 7, 9, 15 and 24 h. Antipyrine was assayed by a spectrophotometric method (Brodie, Axelrod, Soberman & Levy, 1949). The half-life (t0.5) and theoretical plasma concentration at time zero (CP0) were determined by least-square regression analysis of log plasma concentration against time. Antipyrine clearance was calculated (Rowland, Benet & Graham, 1973) as

\[
\text{Clearance} = \frac{\text{Dose}}{\text{CP}_0 \times t_{0.5}}
\]

Results

Total plasma lipids, plasma VLDL and LDL lipids, and plasma VLDL and LDL protein

Initial concentrations of total plasma cholesterol and triglycerides (Tables 1 and 2) were in the normal range for our laboratory. Plasma VLDL triglycerides and plasma LDL cholesterol and triglycerides (Tables 1 and 2) were in the normal range found by Lewis, Chait, Wootton, Oakley, Krikler, Sigurdson, February & Birkhead (1974) for a healthy London population. Nine subjects showed an increase in both total plasma cholesterol and plasma LDL cholesterol after phenobarbitone administration (Table 1) and this was statistically significant for the whole group. Increases in total plasma cholesterol both in individual subjects and the whole group were closely

<table>
<thead>
<tr>
<th>Table 1. Changes in total plasma cholesterol and plasma LDL lipids and protein after oral administration of phenobarbitone (180 mg daily for 3 weeks to ten normal subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean values±SEM are shown. Comparison of results was by Student's paired t-test. NS= not significant.</td>
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<tr>
<td></td>
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<tr>
<td>Before phenobarbitone</td>
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<tr>
<td>Total plasma cholesterol (mmol/l)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
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<tr>
<td>LDL triglycerides (mmol/l)</td>
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<tr>
<td>LDL protein (g/l)</td>
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<tr>
<td>LDL cholesterol:LDL protein</td>
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<td>LDL triglycerides:LDL protein</td>
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</table>
Phenobarbitone and plasma lipids

Table 2. Changes in total plasma triglycerides and plasma VLDL lipids and protein after oral administration of phenobarbitone (180 mg daily for 3 weeks to ten normal subjects)

Mean values ± SEM are shown. Comparison of results was by Student's paired t-test. NS = not significant.

<table>
<thead>
<tr>
<th></th>
<th>Before phenobarbitone</th>
<th>After phenobarbitone</th>
<th>Absolute change</th>
<th>Change (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma triglycerides (mmol/l)</td>
<td>0.94 ± 0.06</td>
<td>1.03 ± 0.09</td>
<td>+0.09 ± 0.07</td>
<td>+11 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL triglycerides (mmol/l)</td>
<td>0.51 ± 0.06</td>
<td>0.53 ± 0.05</td>
<td>+0.02 ± 0.06</td>
<td>+12 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/l)</td>
<td>0.31 ± 0.03</td>
<td>0.33 ± 0.03</td>
<td>+0.02 ± 0.04</td>
<td>+13 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL protein (g/l)</td>
<td>0.12 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>+0.01 ± 0.01</td>
<td>+14 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL triglycerides:VLDL protein</td>
<td>4.33 ± 0.42</td>
<td>4.08 ± 0.37</td>
<td>-0.25 ± 0.49</td>
<td>-6 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL cholesterol:VLDL protein</td>
<td>2.65 ± 0.25</td>
<td>2.60 ± 0.32</td>
<td>-0.05 ± 0.31</td>
<td>-2 ± 12</td>
<td>NS</td>
</tr>
</tbody>
</table>

Similar to increases in plasma LDL cholesterol. Plasma VLDL cholesterol concentrations did not change (Table 2). Eight subjects showed a rise in total plasma triglycerides (Table 2) and six an increase in plasma VLDL triglycerides (Table 2) but the changes were not significant for the group. There was, however, a significant increase in plasma LDL triglycerides (Table 1).

There was a significant increase in plasma LDL protein concentration (Table 1) but not in plasma VLDL protein concentration (Table 2). The ratio of plasma LDL cholesterol to protein was significantly reduced after phenobarbitone administration whereas the ratios of VLDL cholesterol to protein, of VLDL triglyceride to protein and of LDL triglyceride to protein were unaltered.

**Antipyrine clearance**

Absorption of antipyrine was rapid and peak plasma concentrations were obtained in all subjects within 1 h of ingestion. Phenobarbitone induced an increase in the antipyrine clearance of all subjects. The mean initial antipyrine clearance was 43 ± 5 ml/min (± SEM) and after phenobarbitone was 78 ± 8 ml/min, a mean increase of 90 ± 14%. There was no correlation between the alterations in plasma lipids and in antipyrine clearance irrespective of whether these were expressed as absolute or percentage change.

**Serum γ-glutamyl transpeptidase**

Initial serum activities of the enzyme were all within the normal range for our laboratory, the mean value for the group being 12 ± 2.0 i.u./l (± SEM). There was a significant increase after phenobarbitone administration to a mean value of 26 ± 8.0 i.u./l, a mean increase of 95 ± 33%. In two subjects the activities were elevated out of the normal range. There was no correlation between the changes in serum γ-glutamyl transpeptidase and any of those which occurred in plasma lipids. Changes in serum γ-glutamyl transpeptidase were also unrelated to changes in antipyrine clearance.

**Serum phenobarbitone**

Concentrations were in the range 16–27 μg/ml (mean 20.5 μg/ml), confirming that all subjects were taking the phenobarbitone. There was no correlation between serum phenobarbitone concentrations and changes in lipids or changes in antipyrine clearance.

**Discussion**

The present study has shown a significant increase in total plasma cholesterol due to an increase in plasma LDL cholesterol in normal young subjects taking phenobarbitone for 3 weeks. Similar rises in total serum cholesterol during phenobarbitone administration for periods of up to 18 days have previously been reported in three out of four normal subjects (Miller & Nestel, 1973). As regards a possible mechanism for these findings, increased hepatic cholesterol synthesis has been demonstrated in phenobarbitone-treated hamsters (Jones & Armstrong, 1965) and rats (Middleton & Isselbacher, 1969; Wada, Hirata & Sakamoto, 1967). This probably results from induction of the microsomal enzyme 3-hydroxy-3-methylglutaryl-CoA-reductase, which is a rate-limiting step in cholesterol bio-
synthesis (Siperstein, 1970). Phenobarbitone is a non-specific inducer of microsomal enzymes and a relationship might be expected between increases in antipyrine clearance and in cholesterol synthesis if both are due to induction of microsomal enzymes. There was, however, no significant relationship between changes in plasma cholesterol and antipyrine clearance in the present study. The plasma cholesterol concentration is not, though, solely related to the rate of cholesterol synthesis, but also to such factors as accumulation within tissue pools, degradation and elimination, which may also be altered by phenobarbitone. Thus, for example, it has been demonstrated in monkeys receiving this drug that bile salt secretion is increased (Redinger & Small, 1973), and in rats that 7-α-hydroxylation of cholesterol, an important step in bile salt synthesis, is increased after phenobarbitone administration (Wada, Hirata, Nakao & Sakamoto, 1968; Shefer, Hauser & Mosbach, 1972).

In our subjects, the increase in LDL lipids and protein was not accompanied by significant changes in VLDL lipids and protein. This finding is compatible with the currently held view that VLDL is a precursor of LDL (Levy, Bilheimer & Eisenberg, 1971; Eisenberg, Bilheimer, Levy & Lindgren, 1973) since, for example, the turnover of VLDL might be increased without a rise in plasma VLDL concentration, or alternatively a reduction in the rate of removal of LDL from the circulation might occur. Other situations are recognized in which LDL is raised with normal or only modestly elevated VLDL such as type IIa hyperlipoproteinaemia, in which a disorder in feedback inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase has recently been implicated (Goldstein & Brown, 1975).

A further observation in the present study was the proportionately greater rise in LDL protein and triglycerides than that of cholesterol. Knowledge of the factors regulating the apolipoprotein and lipid composition of lipoproteins is, however, at present inadequate (Fredrickson, 1974) to allow further speculation about this finding.

A correlation between the concentration of serum triglycerides and activity of serum γ-glutamyl transpeptidase within the general population has been reported (Martin et al., 1975), the highest values of both occurring in patients with type IIb and type IV hyperlipoproteinaemia in whom the raised triglycerides are in the VLDL fraction of plasma. It was suggested that the raised serum γ-glutamyl transpeptidase reflected increased hepatic microsomal enzyme activity in these subjects and that induction of a microsomal enzyme on the biosynthetic pathway for triglycerides was responsible for the elevated plasma triglycerides. However, other investigators have not accepted serum γ-glutamyl transpeptidase as a quantitative index of microsomal enzyme induction (Rosalki, Tarlow & Rau, 1971; Ideo, de Franchis, del Ninno & Dioguardi, 1971; Rosalki & Rau, 1972), and in the present study changes in serum activity of the enzyme were not related to the changes in antipyrine clearance. We found no significant rise in the total plasma triglycerides or plasma VLDL triglycerides after phenobarbitone administration for 3 weeks, by which time microsomal drug-metabolizing enzymes had been induced. Thus our results do not support the view that there is a simple relationship between plasma VLDL triglycerides and microsomal enzyme induction.

The elevation in plasma lipids observed in this study would probably constitute only a slightly increased risk of coronary artery disease in normal subjects. However, the effect of phenobarbitone over longer periods in older subjects or subjects who have other risk factors for vascular disease may be of importance.

Acknowledgments
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References
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