PLASMA LIPOPROTEINS OF TURKEYS INJECTED WITH A SINGLE DOSE OF DIETHYLSTILBESTROL

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SUMMARY

Turkeys injected with a single dose of diethylstilbestrol (DES) (15, 30 or 60 mg) develop a hyperlipoproteinemia which is characterized by a large increase in chylomicrons, a weak pre-beta lipoprotein concentration and an absence of beta- and alpha-lipoproteins. The hyperlipoproteinemia was pronounced between 3 and 12 days post injection. The rate and degree of development of the induced hyperlipoproteinemia is similar for the 3 levels of DES injected. Increased concentrations of cholesterol and protein are found in the low-density lipoproteins (LDL) between 4 and 20 days post injection, while the cholesterol and protein content of the high-density lipoproteins (HDL) are greatly reduced. The levels of cholesterol and protein in the LDL and HDL return to normal on day 44 post injection.

Key words: Cholesterol – Diethylstilbestrol – Electrophoresis – Lipoproteins – Ultracentrifugation

INTRODUCTION

Turkeys treated with several weekly injections of diethylstilbestrol (DES) succumb from aortic dissecting aneurysms and rhexis as a result of the development of aortic atherosclerosis1,2. The composition of the blood plasma of such DES-treated turkeys is profoundly altered when compared with untreated turkeys. These changes include: hypercalcemia, hypercholesterolemia, hyperproteinemia and hyperlipemia3,4. Recently, it was reported that turkeys injected with a single dose of DES, develop aortic atherosclerosis and a light and electron-microscopic study of the progressive
development of mild to severe atherosclerosis, 4 hours to 44 days post injection of DES, has been made. It was noted in this investigation that turkeys develop grossly altered plasma as early as 2 days following one injection of DES.

In a previous study, turkeys treated with 7 weekly injections of DES, in contrast to non-treated turkeys, developed an altered plasma lipoprotein pattern: a large increase in chylomicrons, a faint pre-beta band and a complete absence of beta- and alpha-lipoproteins. In the present study, involving injections of turkeys with a single dose of DES in varying amounts, it was sought to define the sequential changes that occur in concentration and composition of the major classes of plasma lipoproteins at 1 to 44 days following a single treatment with DES.

MATERIALS AND METHODS

Broad-Breasted White Male Turkeys, 9 weeks old, were injected with a single dose of DES as explained in a previous publication. In the first experiment, 22 turkeys were each given a single injection of 60 mg DES. Six turkeys were retained as controls. After an overnight fast, 5 ml of blood from each of 3 turkeys in the treated and control groups were collected into tubes containing EDTA as anticoagulant. When 2 blood samples were obtained on the same day, the first was taken at 8.00 a.m. after a 12 h fast and the second at 5.00 p.m. after a 6 h fast. Blood samples were taken 2 days before injection, on days 1 (i.e. day of injection), 2 and 3 (2 samples each day) and on days 4, 5, 8, 11, 15, 23, 30 and 37 post injection.

In the second experiment, three groups of turkeys, 30 birds per group, were injected with 15, 30 and 60 mg DES, respectively. Thirty untreated turkeys served as controls. After an overnight fast, 5 ml of blood from each of 3 turkeys in each treatment regimen were collected into tubes containing EDTA as anticoagulant on days 1, 2, 3, 4, 5, 12, 20, 24, 27, 31, and 44 post injection.

The plasma samples obtained at each bleeding from the 3 turkeys in each treatment group were pooled. Lipoproteins of the fresh pooled plasma samples were separated electrophoretically on polyacrylamide gels by the method of Frings et al.; the method uses a separating gel, a stacking gel and a loading gel in a discontinuous buffer system. A 25-μl plasma sample was prestained with Sudan black B in the loading gel prior to electrophoresis. The gels, still inside their glass tubes, were photographed immediately at the end of the electrophoretic run.

Sequential preparative ultracentrifugation was used to isolate lipoprotein fractions in the following two density ranges; D < 1.063 g/ml, low density, and D 1.063–1.210, high density. Seven ml of plasma in a polyallomer tube was overlayered with 4 ml of NaBr solution such that the final solvent density was 1.063 g/ml; it was centrifuged at 4° for 22 h at 105,000 × g (measured at top of tube) in a Type 50 rotor in a Spinco Model L preparative ultracentrifuge. After removal of the top of the tube with a tube slicer, the infranatant portion (retained in the lower section of the tube) was adjusted to give a solvent density of 1.210 g/ml by adding NaBr and centrifuged as before.
The two lipoprotein fractions obtained, low density, \( D < 1.063 \), and high density, \( D 1.063-1.210 \), were purified by resuspending the fraction in \( \text{NaBr} \) solution of appropriate density and centrifuging for 44 h at 105,000 \( \times g \).

Total plasma cholesterol was determined by the method of Levine and Zak. Nitrogen determinations followed the micro Kjeldahl procedure; the factor of 6.25 was used to convert N content to protein concentration.

RESULTS

A single injection of DES (60 mg) rapidly induced profound changes in turkey plasma lipoproteins. The electrophoretic patterns (Fig. 1) make clear the transition from the normal pattern at time of injection to an abnormal pattern at 33 h post injection. The principal change noted 9 h post injection, was an increase in beta- and a slight increase in pre-beta-lipoproteins. At 24 h, the beta- and pre-beta-lipoproteins formed a combined strong band while some weakening of the alpha-lipoprotein band was evident. At 33 h, chylomicrons appeared, alpha-lipoproteins were absent, the beta-lipoprotein band was very weak and the pre-beta-lipoprotein band was still evident. At 48 h post injection only chylomicrons and pre-beta-lipoproteins were present in the electrophoretic pattern. The hyperchylomicronemia persisted until at least day 23 post injection. That chylomicrons were greatly diminished on days 30 and 37 post injection was evidence that the transition to a normal lipoprotein pattern, though not complete, was underway at that time.

Fig. 1. Electrophoretograms of plasma lipoproteins from control and DES-injected turkeys (60 mg). The 25-\( \mu l \) plasma samples were prestained with Sudan black B and run on 3 % polyacrylamide gels. Chylomicrons, if present, form a granular pattern in the loading gel e.g. 33 h through day 23 gels. The dense appearance in the loading gel of preinjection and control plasma is due to residual Sudan black B stain.
All 3 dose levels of DES (15, 30 and 60 mg) induced similar changes in plasma lipoproteins as indicated by polyacrylamide electrophoretic analysis. Fig. 2 shows the lipoprotein patterns obtained with the experimental group of turkeys given 15 mg DES.

Plasma levels of protein and cholesterol and the distribution of these components in the LDL (chylomicrons, beta and pre-beta) and the HDL (alpha) from pooled plasma samples taken on days 4, 20 and 44 post injection with the 3 levels of DES are shown in Tables 1 and 2. Plasma protein and cholesterol levels were increased by approximately the same amount in all 3 groups of turkeys and these increases persisted.

### TABLE 1

**Protein Concentration (mg/ml) in Whole Plasma, Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL) of Control and DES-Injected Turkeys on Days 4, 20 and 44 Post Injection**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DES-treated turkeys</th>
<th><strong>day 4</strong></th>
<th><strong>day 20</strong></th>
<th><strong>day 44</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>15*</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Whole plasma</td>
<td>35.63</td>
<td>51.25</td>
<td>43.13</td>
<td>52.50</td>
<td>43.75</td>
</tr>
<tr>
<td>LDL</td>
<td>1.03</td>
<td>1.75</td>
<td>3.13</td>
<td>3.44</td>
<td>1.31</td>
</tr>
<tr>
<td>HDL</td>
<td>3.95</td>
<td>0.94</td>
<td>1.01</td>
<td>0.82</td>
<td>1.94</td>
</tr>
</tbody>
</table>

*a 15, 30 and 60 refer to the 3 dose levels of DES injected; 15, 30 and 60 mg DES respectively.*
TABLE 2

TOTAL CHOLESTEROL CONCENTRATION (mg/ml) IN WHOLE PLASMA, LOW-DENSITY LIPOPROTEIN (LDL) AND HIGH-DENSITY LIPOPROTEIN (HDL) OF CONTROL AND DES-INJECTED TURKEYS ON DAYS 4, 20 AND 44 POST INJECTION

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DES-treated turkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 4</td>
<td>day 20</td>
</tr>
<tr>
<td></td>
<td>15⁰   30  60</td>
<td>15⁰   30  60</td>
</tr>
<tr>
<td>Whole plasma</td>
<td>1.40  2.80  3.15</td>
<td>2.31  2.60  2.60</td>
</tr>
<tr>
<td>LDL</td>
<td>0.31  1.96  1.90</td>
<td>1.66  1.84  0.90</td>
</tr>
<tr>
<td>HDL</td>
<td>0.88  0.34  0.30</td>
<td>0.64  0.52  0.45</td>
</tr>
</tbody>
</table>

* 15, 30 and 60 refer to the 3 dose levels of DES injected; 15, 30 and 60 mg DES respectively.

until at least day 20 post injection. The concentration of protein and cholesterol in LDL was increased over normal levels while the concentration of these components in HDL was decreased.

DISCUSSION

The present preliminary studies upon the effects of a single injection of DES upon plasma lipids and lipoproteins of turkeys, indicated that this treatment produced a hypercholesterolemia and hyperproteinemia which reached a peak at 4 days post injection. Ultracentrifugation of plasma lipoproteins into LDL (D < 1.063 g/ml) and HDL (D 1.063–1.210 g/ml) indicated that, at 4 days post DES injection, the total cholesterol and protein concentrations in the LDL fraction were increased over control values while the same components in the HDL fraction were decreased. When plasma samples taken on day 4 post injection were separated into their plasma lipoprotein classes by electrophoresis on polyacrylamide gels it was found that chylomicrons were strongly evident, a pre-beta (VLDL) band was evident, however, the beta (LDL) and alpha (HDL) lipoprotein bands were either very weak or absent.

The absence of a stainable band for HDL in the electrophoretograms while the HDL fraction isolated by ultracentrifugation had protein present would seem anomalous. It would appear that the results obtained by ultracentrifugation on the one hand and these obtained by electrophoresis on the other are contradictory. This is not so, however, and can be explained as follows. The electrophoretic technique used in this study involved pre-staining of the plasma sample with Sudan black B. This is a stain for neutral fats, which include triglycerides, phospholipids (lecithins and cephalins) and esterified cholesterol^{10}. The absence of a stainable band in the HDL position on the electrophoretograms, yet the presence of both protein and cholesterol, in the HDL ultracentrifugal fraction (albeit diminished when compared to normal plasma values) would seem to indicate that the apoprotein A had no neutral lipid associated with it and that the cholesterol present was not esterified.
Furman\textsuperscript{11} has reviewed the effect of gonadal hormones upon human serum lipid and lipoprotein patterns. In general, estrogen administration increased the lipid content of both HDL (mainly phospholipid) and VLDL (mainly triglyceride). The response of serum cholesterol levels to estrogen administration was variable and was principally dependent upon the degree and direction of change in the cholesterol content of the LDL. Further, in human familial hypercholesterolemia, estrogen administration had a hypocholesterolemic effect.

The present work and that of Simpson et al.\textsuperscript{3,4} showed that the estrogenic substance DES when administered to turkeys caused a sharp increase in plasma cholesterol levels and that this increase occurred only in the LDL (6–7 times greater than normal levels) while the cholesterol level in the HDL actually decreased (2–3 times lower than normal levels). The administration of estrogen to humans diminished the cholesterol/protein ratio of alpha-lipoproteins (HDL) and also diminished to a lesser extend the ratio of these components in the beta-lipoproteins (LDL). In the present work this ratio in turkeys is increased in both the LDL and HDL with the greatest increase being found with the LDL.

Further studies involving ultracentrifugation of DES induced hyperlipemic plasma into chylomicrons, VLDL, LDL, and HDL fractions and the levels of triglycerides, phospholipids and free and esterified cholesterol within these fractions will provide further insight into the effect of this powerful estrogenic substance upon turkey plasma lipids and lipoproteins.

REFERENCES


