Effects of Dexamethasone on Experimental Atherosclerosis in Cholesterol-fed Rabbits

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Summary We studied the effects of a synthetic adrenocortical steroid, dexamethasone, on the development of experimental atherosclerosis in cholesterol-fed rabbits. Daily intramuscular injection of dexamethasone (0.125 mg/day) remarkably inhibited the aortic atherosclerosis induced by feeding a 1% cholesterol-rich diet for 8 weeks, although it aggravated diet-induced hyperlipidemia. Histologically, less foam cell accumulation was observed in the atherosclerotic lesions of the dexamethasone-treated rabbits as compared with the control animals. When rabbits were fed a normal chow diet for 10 weeks after receiving the 1% cholesterol-rich diet for 8 weeks, no regression of atherosclerotic lesions was observed with the daily injection of dexamethasone (0.125 mg/day); however, the drug again tended to inhibit further progression of atherosclerosis. The anti-atherogenic mechanism of dexamethasone may involve an inhibition of recruitment of blood monocytes and the insudation of atherogenic lipoproteins, mainly β-very low density lipoprotein (β-VLDL) in the present experiments, into the aortic intima, or it may involve a change in the size and structure of the lipoproteins, resulting in their decreased passage through the aortic endothelium into the intima.

Key Words dexamethasone, experimental atherosclerosis, cholesterol-rich diet, hyperlipidemia, monocyte, foam cell, β-very low density lipoprotein, rabbit

Hypercholesterolemia is one of the most important risk factors for atherosclerosis (1, 2). Although cholesterol feedings are sufficient to produce atherosclerotic changes in susceptible animal species such as rabbits and monkeys, in atherosclerosis-resistant species such as dogs and rats it is necessary to use other approaches, among them the production of mechanical injury to the endothelium,
the induction of hypertension, and the administration of anti-thyroid drugs, vitamin D, or foreign proteins (3). Although rabbits are relatively resistant to spontaneous atherosclerosis, the addition of 1 g of cholesterol to the daily diet produces extensive atherosclerotic lesions in the aorta within a few months (4).

In 1952, Oppenheimer and Bruger (5) showed that daily injections of cortisone inhibited the development of atherosclerosis in cholesterol-fed rabbits. An anti-atherosclerotic effect was evident despite the exacerbation by cortisone of the diet-induced hyperlipidemia. Bailey and Butler (3) then reported that all of the steroids they examined, including fluorohydrocortisone, dexamethasone, methylprednisolone, triamcinolone, prednisone and cortisone acetate, were similarly effective. This protective effect could be partially duplicated by various non-steroidal anti-inflammatory drugs including flufenamic acid, phenylbutazone, oxyphenylbutazone and mefenamic acid, although aminopyrine and aspirin were ineffective. Makheja et al. (6) recently reported that cortisone acetate (5 mg daily for 3 months) reduced the formation of atherosclerotic plaques in Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model for familial hypercholesterolemia, although its administration increased plasma levels of cholesterol and triglycerides.

In this study, we confirmed the inhibitory effect of dexamethasone on experimental atherosclerosis in cholesterol-fed rabbits and examined the effect of this steroid on the regression of cholesterol-induced atherosclerosis. We also discuss the anti-atherosclerotic mechanism of steroids.

MATERIALS AND METHODS

Study design. Experiment 1 (Fig. 1): A total of 20 male New Zealand White (NZW) rabbits were selected according to body weight and serum lipid levels to form a homogenous group, and were then randomly allocated into study groups D and C. Both groups received an atherogenic diet (1% cholesterol-rich diet, 100 g/day) for 8 weeks. Group D received 0.125 mg of dexamethasone (in 0.1 ml vehicle) injected intramuscularly every day except Sunday for 8 weeks. The dose of dexamethasone was determined according to Bailey and Butler (3). Group C, the control group, received the same volume of vehicle (0.5 mg sodium bisulfite, 1.5 mg methyl p-hydroxybenzoate, 0.2 mg propyl p-hydroxybenzoate, and 8.0 mg creatinine in 1 ml of distilled water) during the 8-week period. One rabbit in group C died during the study and was excluded from analysis. The animals were sacrificed and autopsied after 8 weeks.

Experiment 2 (Fig. 1): A total of 26 male NZW rabbits were fed a 1% cholesterol-rich diet (100 g/day) for 8 weeks to induce atherosclerosis. The 26 rabbits were then divided into 3 groups so there would be no significant difference in serum total cholesterol levels. Group 1 (N=8) was sacrificed at 8 weeks. Groups 2 and 3 (N=9, respectively) were fed normal chow (100 g/day) for an additional 10 weeks, the normal chow period, and were then sacrificed. During the
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EXPERIMENT 1

1% CHOLESTEROL-RICH DIET (100g/day)  

BASELINE

GROUP 1 (VEHICLE IM) (N=9)

GROUP 2 (DEXAMETHASONE 0.125mg/day IM) (N=10)

8 WEEKS

EXPERIMENT 2

1% CHOLESTEROL-RICH DIET (100g/day)  

BASELINE

GROUP 1 (N=8)

GROUP 2 (VEHICLE IM) (N=9)

GROUP 3 (DEXAMETHASONE 0.125mg/day IM) (N=9)

18 WEEKS

ATHEROSCLEROSIS INDUCTION PERIOD

8 WEEKS  

NORMAL CHOW PERIOD

10 WEEKS

Fig. 1. Design of Experiments 1 and 2.

10-week period, group 3 received daily intramuscular injections of dexamethasone, 0.125 mg, in 0.1 ml of vehicle, while group 2 received vehicle only by injection.

Dexamethasone sodium phosphate solution for injection (Decadron®) was kindly provided by Banyu Pharmaceutical Co. (Tokyo, Japan).

Morphological analysis. The aortas were incised longitudinally, their internal surfaces were photographed, and the area of atherosclerotic plaques and the total area of the aortic surfaces were calculated with a computerized planimeter (Digitizer Model No. DT 1000, Watanabe Ins. Corp., Japan). The relative deposition of plaque was expressed as the proportion of plaque area to total aortic area × 100 (%). (4)

Histological analysis. The aortas were fixed in 10% aqueous formaldehyde solution. Sections from the aortas were embedded in paraffin, sectioned and stained with hematoxylin-eosin and elastica van Gieson.

For lipid staining, part of the aortas were fixed in 95% ethanol and stained with Sudan III.

Laboratory determinations. Serum lipid levels were measured by the following methods. Total cholesterol, triglycerides, and phospholipid were determined by
enzymatic method kits (Nippon Shoji, Japan) and HDL-cholesterol by a heparin-Ca method kit (Nippon Shoji, Japan). Very low density lipoprotein (VLDL) was isolated by ultracentrifugation (7).

Statistical analysis. Results were expressed as mean ± SE unless otherwise stated. Statistical evaluation was done using the Student’s t-test, with a p value of <0.05 considered to be statistically significant.

RESULTS

Experiment 1

Body weight and serum lipids: Increase in body weight were suppressed in group D as compared with group C after 3 weeks (Fig. 2). Serum cholesterol, triglyceride and phospholipid levels were higher in group D than in group C at the end of the experiment (Fig. 3). Serum levels of VLDL-cholesterol (C), VLDL-triglycerides (TG), and VLDL-phospholipid (PL) were significantly higher in group D (Table 1). The ratio of VLDL-C/VLDL-TG was significantly lower in group D than in group C, suggesting that the VLDL particles in group D were richer in TG. The weight of the right femur was slightly less in group D than in group C, but not to a statistically significant extent (8.50 ± 0.15 g vs. 9.07 ± 0.26 g, p < 0.1).

Atherosclerotic involvement of the aorta was evaluated by computerized planimetry, with the results expressed as a percentage of the total aortic surface covered by fatty lesions (Fig. 4). In Group C, 40.0 ± 3.7% of the aortic surface was affected whereas in group D, only 2.8 ± 0.9% of the aortic surface was involved, a highly significant difference (p < 0.001).

Histological examination of the aorta revealed early atherosclerotic lesions characterized by the accumulation of foam cells, full of lipid droplets stained with Sudan III, in the intima. Figure 5 shows representative aortic sections from groups

![Fig. 2. Changes in body weight in Experiment 1. Results are expressed as mean ± S.D. *p < 0.05, **p < 0.01.](image)
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**Fig. 3.** Serum levels of total cholesterol, HDL-cholesterol, triglycerides, and phospholipid in Experiment 1. *p < 0.01; NS, not significant.

**Table 1.** Lipid composition of the VLDL fraction in Experiment 1.

| Lipid Composition       | Group C (mg/dl) | Group D (mg/dl) |
|-------------------------|-----------------|-----------------|
| VLDL-C                  | 1,563±171       | 2,496±190*      |
| VLDL-TG                 | 503±82          | 1,420±202*      |
| VLDL-PL                 | 1,141±165       | 2,275±163*      |
| VLDL-C/VLDL-TG          | 3.61±0.40       | 1.99±0.27*      |

*p < 0.01.

C and D. A decrease in lesion thickness and in cell components, particularly foam cells, was evident in group D as compared with group C.

**Experiment 2**

There was no significant difference in body weight between the 3 groups until 8 weeks, although at 18 weeks, the body weight of group 2 (vehicle-treated) was significantly greater than that of group 3 (dexamethasone-treated) (Fig. 6). Serum total cholesterol levels increased until week 8, then gradually declined after the animals changed to a normal diet, approaching baseline values by the end of the experiment (Fig. 7). Serum triglyceride levels showed similar changes (data not...
The progression of atherosclerosis tended to be inhibited in group 3 as compared with group 2, although this finding was not significant. However, no regression was observed in either group during the administration of regular chow for 10 weeks, the normal chow period (Figs. 8, 9).
DISCUSSION

Adrenocortical steroids have been used clinically to treat a wide variety of diseases, among them collagen-vascular diseases, nephrotic syndrome and asthma, and are often administered for prolonged periods. There is concern that long-term therapy increases the risk of coronary artery disease (8) because these agents induce a rise in serum lipids, especially cholesterol, due to an elevation of LDL (9). Kinetic studies of LDL have indicated both an increased flux of apo B into LDL and a decrease in the fractional catabolic rate of LDL after adrenocortical steroid administration (9).

The present study investigated the effects of dexamethasone on the development of atherosclerotic lesions in the aorta of cholesterol-fed rabbits. In two experiments, we found that dexamethasone protected against the progression of...
Fig. 8. Aortic templates in Experiment 2. Shaded areas correspond to atherosclerotic lesions.

Fig. 9. Aortic atherosclerosis involvement (%) in Experiment 2. NS, not significant.
atherosclerotic lesions, although it did not induce a regression of previously formed lesions. However, the 10-week regression period in Experiment 2 may have been too short to allow the regression of formed lesions since it is known that the atherosclerotic lesions induced in rabbits by cholesterol feeding are relatively resistant to such a diet (10, 11). It should be pointed out that dexamethasone inhibited the development of atherosclerosis despite increased serum lipid levels.

Dexamethasone administration aggravated the hyperlipidemia induced by diet in this study. If hyperlipidemia is associated with a change in lipoprotein structure or size that affects uptake by macrophages, this effect of dexamethasone should protect against the development of atherosclerosis (12). In severe hypertriglyceridermic conditions such as those seen in diabetes, the occurrence of large lipoproteins may lead to a reduction in atherosclerosis due to steric effects (13, 14). The above-mentioned aspects of dexamethasone are presently being investigated in this laboratory.

Adrenocortical steroids such as dexamethasone exert a variety of pharmacologic actions (15), four of which may be involved in the observed anti-atherogenic effect: (1) inhibition of the synthesis of arachidonic acid metabolites including the prostaglandins and leukotrienes involved in inflammatory processes; (2) inhibition of the increase in vascular permeability and plasma infiltration during the initial period of inflammation; (3) stabilization of the cell membrane; (4) inhibition of the adhesion of polymorphonuclear leukocytes and monocytes to the vascular endothelium and of the infiltration to the site of inflammation.

It has been shown that foam cells in atherosclerotic lesions largely originate from circulating monocytes (16, 17). Adrenocortical steroids are known to induce prolonged monocytopenia (18). In fact, the characteristic reduction of mononuclear phagocytes at the site of inflammation during steroid treatment has been explained by a reduction in blood monocytes (18). In this study, the blood monocyte count could not be accurately determined because of the extremely high serum lipid levels (data not shown). Another possibility is that dexamethasone may have modulated the lipoprotein metabolism of the monocyte/macrophages, inhibiting the activity of the LDL receptor and stimulating that of the acetylated LDL receptor in the macrophages (19).

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