Effects of Ovariectomy on Experimental Atherosclerosis in Rabbits

By GIUSEPPE MININNI, M.D., STEPHEN CONTRO, M.D. AND CARLO CECCHIA, M.D.

The effect of previous ovariectomy on the development of cholesterol-induced atherogenesis in the rabbit was investigated. It was found that an ovariectomized group of animals had a significantly greater degree of hypercholesterolemia, but that the control group with intact ovaries developed slightly more aortic and coronary atheromatosis. The implications of these findings are discussed.

Recently, evidence has been obtained in man and chick supporting the concept that estrogens play a key role in the well known resistance of pre-menopausal females to coronary atherosclerosis. The present investigation was undertaken to explore this problem further in rabbits. In essence, a comparison was made of cholesterol-induced aortic and coronary atherogenesis in intact versus ovariectomized female rabbits, in order to ascertain whether physiologic estrogen secretion inhibited cholesterol-induced coronary atherogenesis in this mammalian species.

Methods

The study utilized 29 female rabbits of the same strain, approximately 6 months of age and 1.7 Kg. in initial weight. The animals were divided into two groups: Group 1 was composed of 15 unoperated control animals, Group 2 of 14 rabbits surgically castrated under sodium pentothal anesthesia. Surgical examination revealed hemorrhagic ovarian follicles, confirming the presence of active ovarian function. Ten days after surgery both groups were placed on a diet of mash (50 Gm./rabbit/day), supplemented with a daily intake of 0.5 Gm. of cholesterol, dissolved in 5 cc. of olive oil. Ample greens (lettuce and cauliflower) were also fed daily. Blood samples were taken every two weeks and analyzed for total cholesterol and lipid phosphorus. All rabbits were sacrificed on the 50th experimental day. At autopsy the aortas were inspected for atherosclerotic plaques, and the lesions were grossly graded from 0 to 4. Three blocks were cut from each heart, which had been fixed in 10% formaldehyde. Two frozen sections were then cut at different levels of each block and stained with Sudan III for lipids. The grading of coronary lesions ranged from 0 to 4 according to the extent and severity of microscopic findings. Slides were indiscriminately mixed and treated as unknowns for grading. The following summarizes the criteria for grading: Grade 0—no lipid-containing intimal lesions; Grade 2—many vessels with lipid material, but no sclerosis, or few vessels with sclerosis and plaques; Grade 3—any lesion greater than 2 but less extensive than 4; Grade 4—all vessels with lipid material and slight to marked thickening of their wall.

Results

Aortic and Coronary Atherogenesis. The ovariectomized rabbits exhibited significantly less gross aortic atherogenesis than the intact controls (table 1). The hearts appeared grossly normal. On microscopic examination all hearts of both groups (except one of Group 2) presented coronary lesions. In accord with the aortic findings, the ovariectomized group exhibited significantly lower grading of coronary lesions (table 2). In most animals of both groups, lesions consisted of subendothelial lipid infiltration. Only two animals in Group 1 and one in Group 2 exhibited atherosclerotic plaques impinging upon the lumen of the artery.

Biochemical Findings. Biochemical and pathological findings did not correlate, in that the ovariectomized rabbits exhibited significantly less aortic and coronary atherogenesis, despite higher plasma cholesterol levels. The difference between the two groups cannot be attributed to any difference in patterns of food intake or weight change, since the two groups were essentially similar in these two respects (table 3).
TABLE 1.—Effects of Ovariectomy on Aortic and Coronary Atherosclerosis in Cholesterol-Fed Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Rabbits</th>
<th>Gross Aortic Atherosclerosis</th>
<th>Microscopic Coronary Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Incidence %</td>
<td>Grade</td>
</tr>
<tr>
<td>1 Control</td>
<td>15</td>
<td>87</td>
<td>1.9 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0-0.4†</td>
</tr>
<tr>
<td>2 Ovariectomized</td>
<td>14</td>
<td>50</td>
<td>0.4 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0-1.5†</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† Range.

TABLE 2.—Effects of Ovariectomy on Plasma Lipids in Cholesterol-Fed Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma Total Cholesterol mg/dl</th>
<th>Plasma Lipid Phosphorous mg/dl</th>
<th>C/P Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>413 ± 34.4†</td>
<td>16.0 ± 1.0†</td>
<td>26.3 ± 2.5†</td>
</tr>
<tr>
<td></td>
<td>200-555†</td>
<td>8.8-21.5†</td>
<td>9.8-30 2†</td>
</tr>
<tr>
<td>2 Ovariectomized</td>
<td>540 ± 23.4†</td>
<td>10.0 ± 1.2†</td>
<td>29.4 ± 1.4†</td>
</tr>
<tr>
<td></td>
<td>375-705†</td>
<td>14.2-31.0†</td>
<td>14.6-38.0†</td>
</tr>
</tbody>
</table>

* C/P ratio is the plasma total cholesterol/plasma lipid phosphorous.
† Standard error of the mean.
‡ Range.

TABLE 3.—Body Weight and Food Intake

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Body Weight kg</th>
<th>Terminal Body Weight kg</th>
<th>Match Intake, mg/rabbit/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>1.6 ± 0.04*</td>
<td>2.1 ± 0.07*</td>
<td>50†</td>
</tr>
<tr>
<td></td>
<td>1.4-1.8†</td>
<td>1.8-2.3†</td>
<td></td>
</tr>
<tr>
<td>2 Ovariectomized</td>
<td>1.9 ± 0.06*</td>
<td>2.1 ± 0.06*</td>
<td>50†</td>
</tr>
<tr>
<td></td>
<td>1.5-2.3†</td>
<td>1.6-2.4†</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean.
† Range.
‡ This amount was consumed in toto by all rabbits throughout the experiment, together with the supplementary cholesterol-oil and greens (see text).

Discussion

The results of this investigation demonstrate that the intact female rabbit with functioning ovaries is no more resistant to cholesterol-induced coronary atherogenesis than her ovariectomized counterpart. They indicate that the endogenous estrogen secretion of the mature female rabbit is not effective in prophylactically inhibiting cholesterol-induced coronary atherogenesis. This finding is in accord with the work showing that exogenous estrogens do not prevent cholesterol-induced lesions in the coronary arteries of this mammalian species. Clearly, these negative results in the rabbit are in significant contrast with findings in the chick, wherein both exogenous and endogenous estrogens are remarkably effective both prophylactically and therapeutically in inhibiting cholesterol-induced coronary atherogenesis.

A few reports are available on the influence of sex and sex hormones on experimental atherosclerosis in the rabbit. Thus it was reported many years ago, and recently confirmed, that intact male rabbits are more resistant to cholesterol-induced atherogenesis than females, a situation opposite to that observed in mature human beings. This sex difference is presumably reversible, since estrogenic treatment renders male rabbits susceptible, and androgen administration makes female animals resistant. Others have reported somewhat different findings, i.e. that both androgens and estrogens protect intact female rabbits against hypercholesterolemia and atherosclerosis. It has also been reported that castration intensifies cholesterol-induced atherogenesis in rabbits. Additional studies apparently are essential to resolve the contradiction in these several reports.

Further work is also necessary to ascertain the basis for the marked species difference in the effect of estrogens on atherogenesis. It may be related to the different plasma lipid response of the two species to the hormone, since the estrogen-treated cholesterol-fed chick exhibits a marked enhancement of phospholipemia, with depression of the plasma C/P ratio toward normal limits, whereas this response is minimal or absent in the rabbit.

In view of this species difference, clinical investigation is essential to determine the effects of estrogens on atherogenesis in man.

Summary

In an attempt to assess the influence of endogenous ovarian secretion on experimental cholesterol-induced atherogenesis, a comparison was made between intact and ovariectomized rabbits.
The two groups of rabbits exhibited the following findings in response to a cholesterol-supplemented diet (0.5 Gm cholesterol in 5 cc. of olive oil daily) fed for 50 days: Similar patterns of feed intake and weight gain; more marked hypercholesterolemia in the ovariectomized group; more marked aortic and coronary atherogenesis in the intact group.

It is concluded that endogenous ovarian secretion in the intact female rabbit has no suppressing influence on experimental cholesterol-induced atherogenesis in either the aorta or the coronary vessels.

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REFERENCES

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