Effect of Carcinogens on Chicken Atherosclerosis

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SUMMARY

Weekly i.m. injections of the polycyclic hydrocarbon carcinogens, 7,12-dimethylbenz(a,h)anthracene (DMBA; 25 mg/kg/injection) and benzo(a)pyrene (50 mg/kg/injection) were given for a period of up to 22 weeks to chickens (SC strain) beginning at age 4 weeks. Atherosclerotic lesions of the abdominal aorta occurred more frequently and were larger in the DMBA- and benzo(a)pyrene-treated birds than in controls. These lesions were proliferative in character as indicated by a higher [3H]thymidine autoradiographic labeling index compared to the underlying medial cells of the aorta. Measurements of serum cholesterol in DMBA-treated birds showed no differences from controls. Although both carcinogens accelerated the development of atherosclerotic plaques, DMBA was more potent than benzo(a)pyrene.

INTRODUCTION

Benditt and Benditt (2) reported that individual abdominal aortic plaques from heterozygotic women contain either one or the other of the 2 isoenzymes of glucose-6-phosphate dehydrogenase, whereas the normal aortic wall from the same individuals contains a mixture of both isoenzymes. This evidence for the monoclonal origin of the atherosclerotic plaques, confirmed by Pearson et al. (6), suggests the possibility that atherosclerotic lesions are benign smooth muscle tumors of the aortic wall and raises concern about the possible role of environmental carcinogens on human atherosclerosis.

The purpose of this study was to examine the question of whether carcinogens enhance the development of atherosclerosis. The chicken was chosen for study because the atherosclerotic plaques that develop spontaneously in the abdominal aorta resemble those that occur in humans (5).

A pilot study showed evidence that DMBA5 in contrast to X-radiation, 2-acetylaminofluorene, and N-methyl-N'-nitro-N-nitrosoguanidine caused an earlier appearance of aortic atherosclerosis in chickens (1). This paper reports additional studies with DMBA and BAP, including the effects of DMBA on serum cholesterol levels and [3H]thymidine autoradiographic cell-labeling indices in the atherosclerotic plaques in relation to the underlying medial cells.

MATERIALS AND METHODS

SC strain chickens (Hy-Line International, Des Moines, Iowa) were given weekly injections in the pectoral muscle of DMBA (25 mg/kg) or BAP (50 mg/kg) dissolved in DMSO beginning at 4 weeks of age. Control groups received either DMSO or no treatment. Birds in the various groups were killed by air embolism at various times between 13 and 18 weeks after the carcinogen treatments were started.

The aorta was removed from each bird starting from the level of the mid thorax down through the abdomen to its bifurcation. The anterior wall of the aorta was opened lengthwise. The aorta was then divided serially into 3-mm segments along the longitudinal axis of the vessel. The aortic segments were imbedded in paraffin, and histological sections were made from each segment. The sections were stained by the Verhoeff-Van Gieson procedure or with hematoxylin and eosin (4). The cross-sectional area of the atherosclerotic plaque on each aortic segment was measured microscopically by use of an eyepiece graticule. The volume of the plaque on each aortic segment was calculated by multiplying the cross-sectional area by 3 mm; the total volume of the plaque was obtained by summing the volumes of each segment.

Serum cholesterol levels were determined by the Ferro-Ham method (3) after 14, 15, and 22 weeks of carcinogen treatment. Plaque and medial cell labeling indices were obtained from each of the groups receiving either DMBA or BAP in DMSO, DMSO, or no treatment after 15 weeks on test. The DMBA-treated and solvent-treated birds were given a single i.v. injection of 0.5 μg [3H]thymidine per g 1 hr before death. The BAP-treated birds were given 6 injections of [3H]thymidine within a 36-hr interval in order to enhance the degree of labeling and were killed 12 hr after the last injection. Autoradiographs prepared by a dipping procedure in NTB-2 (Eastman Kodak, Rochester, N. Y.) of sections were exposed 2 weeks. The proportion of labeled cells in the BAP-treated birds was divided by 6 to compensate for the extra thymidine injections.

RESULTS

Plaques were observed only in the abdominal aorta. These lesions had the same characteristics as described by others (5). The plaques occurring in the carcinogen-treated birds were indistinguishable morphologically from those
that occur at a later time without carcinogen treatment. In each affected aorta, the plaque generally consisted of a single ridge-like structure located mostly on the posterior wall, arising between the orifices of the renal arteries and growing caudally. The lesions were fibrous with a substantial cellular component and little evidence of extracellular lipid accumulation or foam cells. The lesions were located between the internal elastic lamina and the endothelium. A plaque, well labeled with $[^3H]$thymidine, from a BAP-treated bird is shown in Fig. 1.

Results of the study are shown in Table 1. The data for the prevalence of plaques, serum cholesterol levels, and labeling indices are grouped for sacrifice times extending over a period from 13 to 22 weeks, because there was no time dependence on the results within this period. Plaques were scored only if they exceeded 0.5 cu mm in volume. Exposure to the carcinogens produced a marked increase in the prevalence of plaques; only 1 plaque was noted among 19 (6%) birds receiving no treatment, and 2 were noted among 24 (8%) birds receiving the solvent, DMSO. By contrast, BAP- and DMBA-treated birds exhibited plaques in 10 of 15 (66%) and 20 of 28 (72%) cases.

No significant effect of treatment was observed on serum cholesterol values, which averaged 155 mg/100 ml in the nontreated birds and 142 and 138 mg/100 ml, respectively, in the DMBA and DMSO groups.

Labeling indices in the plaques were significantly elevated in comparison to those of the medial cells. The solvent increased the labeling index of the medial cells, but neither carcinogen produced a significant change in the medial cell labeling index when compared to the results for treatment with solvent alone.

The carcinogen treatments retarded the growth of the chickens, as shown by the data in Table 1. Sarcomas were found in the abdominal cavity of 3 DMBA-treated birds.

Chart 1 shows the mean plaque volume (total volume of all plaques per total birds) and the standard errors of the means as a function of time for the groups receiving DMBA, BAP, or DMSO. There was an increase in plaque volume with time in both the DMBA- and BAP-treated birds, reflecting an increase in both plaque prevalence and individual plaque size.

**DISCUSSION**

A high proportion of chickens spontaneously develop large atherosclerotic plaques by 1 year of age. The mechanism of formation of the lesion is not known. The sacrifice times in this study between 13 and 22 weeks were chosen to minimize the occurrence of lesions in the control birds. The increase in prevalence and volume of the atherosclerotic plaques in the birds treated with BAP and DMBA reflects a faster rate of development. The higher labeling index of plaque cells indicates that the plaque growth is a function of

**Table 1**

The effect of carcinogens and solvent on chicken aortas, blood, and weight

Chickens were subjected to weekly i.m. injections of 25 mg DMBA or 50 mg BAP per kg dissolved in DMSO. At various times between 13 and 18 weeks of treatment, birds received injections of $[^3H]$thymidine, and autoradiographs of their aortas were prepared. At various times between 13 and 22 weeks, blood samples taken from DMBA-treated, DMSO-treated, and untreated birds were analyzed for cholesterol. The $p$ values refer to a comparison of plaque cells to medial cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction of birds with plaque &gt;0.5 cu mm $a$</th>
<th>Serum cholesterol (mg/100 ml ± S.E.)</th>
<th>Labeling index $b$ (% ± S.E.)</th>
<th>Weight as % of control at 10 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum cholesterol (mg/100 ml ± S.E.)</td>
<td>Plaque cells</td>
<td>Medial cells</td>
<td></td>
</tr>
<tr>
<td>BAP</td>
<td>10/15 (66)$c$</td>
<td>142 ± 12</td>
<td>0.12 ± 0.02</td>
<td>0.76 ± 0.24 (p = 0.05)$d$</td>
</tr>
<tr>
<td>DMBA</td>
<td>20/28 (72)</td>
<td>138 ± 6</td>
<td>0.12 ± 0.05</td>
<td>IN</td>
</tr>
<tr>
<td>DMSO</td>
<td>2/24 (8)</td>
<td>155 ± 9</td>
<td>0.032 ± 0.007</td>
<td>IN</td>
</tr>
<tr>
<td>None</td>
<td>1/19 (6)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Denominator is the number of birds on test.
* Values for BAP were divided by 6 to compensate for extra injections of $[^3H]$thymidine.
* Numbers in parentheses, percentage.
* NA, not available; IN, insufficient numbers of plaques.
* Based on modified 2-sample $t$ test.

JULY 1977
cell proliferation. DMBA was more potent than BAP, since the plaque volumes and the [3H]thymidine labeling indices were several times greater in DMBA-treated birds even though the dose of DMBA was half as large as that for BAP. DMBA is also a more potent carcinogen than BAP (8). The mechanism of action of the 2 polycyclic aromatic hydrocarbon carcinogens in speeding the process of atherosclerosis in the chickens is not known. There was some generalized toxicity as indicated by loss of weight, although much of the weight loss was attributable to the solvent alone, which did not speed the formation of atherosclerotic lesions.

It is unlikely that the plaques were produced by a carcinogen-mediated alteration in the serum cholesterol levels, because the effect of the DMBA-solvent combination, if anything, was to lower rather than raise serum cholesterol, and the DMBA had no effect at all beyond that produced by the solvent alone. In addition, the plaques noted in the present experiment were exclusively abdominal, whereas cholesterol induces a fatty type of plaque in chickens which tends to be localized to the thoracic region (5, 7).

Although the specific mechanism remains unclear, the results presented here indicate clearly that 2 of the polycyclic hydrocarbon carcinogens produce effects on the atherosclerotic process in chickens that are analogous to the shortened appearance time for spontaneously occurring animal tumors. The mechanism of action of the carcinogens in inducing atherosclerosis warrants further study, especially in light of recent evidence of the monoclonicity of human plaques and older evidence that methylcholanthrene may induce sclerotic lesions of the aorta in mice given a cystine-deficient diet (9).

REFERENCES

Fig. 1. Autoradiograph of a fibromuscular plaque in the abdominal aorta of a BAP-treated chicken. A, section from the distal end of the plaque; B, section from the middle of the same plaque.
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