EXPERIMENTAL STUDIES

Suppression of Experimental Atherosclerosis in Rabbits by Interferon-Inducing Agents

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The effects of two chemically different interferon inducers on the suppression of atherosclerosis were studied in rabbits fed an atherogenic chow diet. One group (10 rabbits per group) was fed normal rabbit chow, and three groups were fed an atherogenic chow. One of the latter groups received the atherogenic feeding alone; the other two were treated with either polyinosinic-polycytidyl acid (poly I:C) or 2-amino-5-bromo-6-phenyl-4-pyrimidinone (ABPP). Neither of the drugs reduced significantly the hypercholesterolemia induced by the feeding. However, both poly I:C and ABPP treatment significantly reduced the percent area of the aortic intimal surface lesions, stained for lipid with Sudan IV, compared with that in untreated rabbits fed atherogenic chow.

The pathogenesis of atherosclerosis is characterized by the proliferation of cells in the intima and the focal accumulation of lipid and connective tissue. Although elevated serum cholesterol is considered to be a major factor in the development of the disease, several effective antiatherogenic agents that are not aimed at lowering serum cholesterol have been described. Most of these, such as ni fedipine, diphosphonates and lanthanum, act by inhibiting the calcium overload in arterial cells of cholesterol-fed rabbits. However, suppression of experimental atherosclerosis in rabbits was also achieved with an antiproliferative drug, colchicine, and its derivative Demecolcine (Colcemid). Interferon is being used to suppress abnormal cellular proliferation in the treatment of malignant diseases. Hypothesizing that the atherogenic mechanism might be similar to neoplastic activity, we initiated studies to examine the possibility of using interferon or its inducers as antiatherogenic agents.

Interferons are glycoproteins produced by many types of cells in response to induction by a chemically diverse group of agents, and exhibit a variety of antiviral, anticancer and immunomodulatory activities. We report on treatment with two chemically unrelated interferon inducers: a high molecular weight synthetic polynucleotide, polyinosinic-polycytidylic acid (poly I:C), and a low molecular weight pyrimidine derivative, 2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (ABPP), which suppress aortic cellular proliferation and lipid deposition in the cholesterol-fed rabbit, without reducing the dietary-induced hypercholesterolemia.

Methods

Animals and diet. Forty young adult male New Zealand white rabbits weighing 2 to 2.5 kg were assigned randomly to four groups of 10 rabbits each. One group received a diet...
of Purina rabbit chow (normal diet). The other three groups received a fibrogenic-atherogenic diet (10).

**Experimental procedure.** One group of 10 rabbits was fed the atherogenic diet without drug treatment (no drug group). The second group was treated with poly I.C (P-L Biochemicals, Inc.) once a week by intravenous injection (100 μg/kg body weight) into the marginal ear vein. The synthetic double-stranded ribonucleic acid (RNA) (poly I C) was reconstituted at a concentration of 1 mg/ml in sterile phosphate-buffered saline solution, pH 7.0 (0.006 M sodium phosphate, 0.15 M sodium chloride) and allowed to re-anneal by heating in a 50°C water bath until dissolved and then standing at room temperature to cool. The third group was treated with ABPP (The Upjohn Company) once a week by intraportal injection in doses of 200 mg/kg body weight. This drug was administered as a suspension in 5 ml sterile phosphate-buffered saline solution using a two-syringe technique to generate and deliver the suspension.

Body weight and food consumption were recorded and fasting blood samples were collected at the beginning of the study and after 4 and 8 weeks of the diet treatment for the determination of total cholesterol by the Technicon AAI automated Liebermann-Burchard method as described in the Lipid Research Clinics Program Manual (11). Lipoproteins were separated from serum by sequential ultracentrifugation (12). Serum interferon titers were determined by a vesicular stomatitis viral plaque reduction assay on primary rabbit kidney cells (13).

**Postmortem studies.** The rabbits were killed after 9 weeks and the whole aorta from the aortic valve to the iliac bifurcation was removed from each animal. The aorta was opened longitudinally along its anterior wall, and the adventitia and adhering adipose tissue were removed. The vessel was fixed and stained grossly with Sudan IV (14) to visualize the lipid-rich atherosclerotic lesions. After tracing on an overlaid clear plastic sheet, the aortic surface area and its lipid-staining lesions were quantitated by an electronic graphics calculator (model 1223, Numonics Corp). Before staining, small samples of aortic tissue were taken from three areas of each aorta, proximal to the left subclavian artery, including the fifth intercostal arteries, and distal to the origin of the celiac trunk. The sample from the thoracic aorta was freeze-sectioned and stained with oil red 0 for neutral lipid. The other two samples of aortic tissue were embedded in paraffin, sectioned and stained routinely with hematoxylin-eosin, Masson's-trichrome stain for collagen and Verhoeff-Van Gieson's stain for elastin. Sections from three areas of each paraffin block were used to quantitate lesion areas with a Zeiss Video-Plan morphometric analysis system. This analysis was performed blindly to avoid subjective bias.

**Assays.** Biochemical analysis of the total cholesterol content of the whole aorta was performed on duplicate aliquots of the total lipid extract (15) of the freeze-dried tissue. Total aortic collagen content was determined by measuring the hydroxyproline content in duplicate using a colorimetric method (16). The statistical significance of intergroup differences was determined by one-way analysis of variance using the Student-Newman-Keuls procedure for pair-wise comparisons (17).

**Results**

**Serum lipids.** There were no significant differences in the average food consumption and weight gain between the normal control group and the experimental groups of rabbits. In the rabbits fed the atherogenic diet, the concentration of total serum cholesterol increased from the normal levels to atherogenic levels whether or not interferon-inducing drugs were administered. The mean total serum cholesterol values (mg/100 ml) for each group after 8 weeks of feeding were 43 ± 15 (mean ± standard deviation) in the normal diet group, 3,270 ± 873 in the untreated cholesterol-fed group and 2,709 ± 970 and 2,580 ± 899 in the cholesterol-fed groups treated with poly I C and ABPP, respectively (Fig 1). The elevated serum cholesterol was due to elevated levels of cholesterol in very low density, intermediate density and low density lipoprotein fractions. Increases in serum triglyceride levels in the rabbits fed the atherogenic diet were smaller than those of serum cholesterol and were confined to very low and intermediate density lipoprotein fractions. There was no significant increase in low density lipoprotein triglyceride content.

**Interferon levels.** Serum interferon levels (3 hours after injection) averaged 351 ± 29 units/ml in rabbits treated with poly I C for 8 weeks compared with an average of 698 ± 28 units/ml after the first injection. This result indicated

![Figure 1](image-url)

**Figure 1.** Mean total serum cholesterol levels in rabbits receiving the atherogenic diet (AD) with or without one of the interferon-inducing drugs, poly I C (polynosinic-polycytidylic acid) or ABPP (2-amino-5-bromo-6-phenyl-4-pyrimidinone), for 8 weeks. The bars indicate the mean values for each group. NS = not significant.
that the weekly spacing of the injections had rather good success in overcoming the development of hyporeactivity (9). No comparable interferon data were obtained for ABPP because of the slow interferon response of this drug when injected intraperitoneally (18).

**Macroscopic findings.** Figure 2 shows representative tracings of one aorta from each group. Rabbits fed the atherogenic diet without drug treatment developed widespread, grossly visible atherosclerosis. Portions of both the thoracic and abdominal aortic surfaces and almost the entire aortic arch were covered with lipid-staining lesions. The percent of the intimal surface affected by lesions was reduced significantly by both poly I C treatment and ABPP treatment (probability \[p\] < 0.05) compared with that in the untreated atherosclerotic group. The fatty lesions involving the abdominal and thoracic aorta appeared to be reduced in both number and size, while the aortic arch lesions were more resistant to suppression. There was no significant difference in the reduction or distribution of the lesions between the two drug-treated groups.

**Biochemical results.** Aorta from rabbits fed the atherogenic diet contained significantly more (\(p < 0.001\)) cholesterol than aorta from the normal diet group (Table 1). Treatment with either poly I C or ABPP resulted in a highly significant reduction (\(p < 0.001\)) in the cholesterol accumulation in the aorta. The reduction in aortic cholesterol accumulation was not significantly different between the two drug-treated groups. The increase in collagen content and dry weight observed in rabbits fed the atherogenic diet was not significantly affected by drug treatment.

**Microscopic findings.** Microscopic examination of frozen sections of thoracic aorta stained with oil red 0 for lipid revealed that compared with normal aorta (Fig 3A), sections from untreated, cholesterol-fed rabbits (Fig 3B) exhibited a markedly raised intima with large proliferations of lipid-laden "foam" cells and some derangement of the intimal-medial elastin layers. In rabbits treated with either of the two interferon inducers (Fig 3C and D), the lesions consisted of fewer layers of intimal foam cells overlaying an otherwise normal arterial wall.

**Table 1.** Effect of Interferon-Inducer Treatment on Cholesterol Accumulation, Collagen Content and Dry Weight of Aorta in Rabbits Fed an Atherogenic Diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/aorta per kg body weight)</th>
<th>Collagen Hydroxyproline (mg/aorta per kg body weight)</th>
<th>Dry Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td>0.08 ± 0.04</td>
<td>1.59 ± 0.31</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Atherogenic diet</td>
<td>1.04 ± 0.24</td>
<td>1.90 ± 0.43</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>No drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atherogenic diet + Poly I C</td>
<td>0.39 ± 0.26*</td>
<td>1.94 ± 0.50</td>
<td>0.17 ± 0.07</td>
</tr>
<tr>
<td>Atherogenic diet + ABPP</td>
<td>0.43 ± 0.26*</td>
<td>1.96 ± 0.36</td>
<td>0.20 ± 0.03</td>
</tr>
</tbody>
</table>

*\(p < 0.001\) compared with atherogenic diet, no drug. ABPP = 2-amino-5-bromo-6-phenyl-4-pyrimidinone, poly I C = polymosinic-polycytidylic acid.

**Figure 2.** Tracings of aorta from rabbits that received either a normal diet or the atherogenic diet with or without one of the interferon-inducing drugs. The black areas represent Sudan IV-positive lipid lesions. The mean (± standard deviation) surface area covered in each group is shown below each representative tracing. *\(p < 0.05\)

**Discussion**

The use of interferon-inducing agents circumvents many of the limitations inherent in the production and isolation of species-specific interferons. In this study, we used two chemically dissimilar inducers—poly I C, a synthetic double-stranded RNA well known to be a potent inducer of rabbit interferon (8), and a newly developed low molecular weight pyrimidine derivative, ABPP (9), which induces high levels of serum interferon in several different species (18). The data presented in this report indicate that both of these drugs were effective in suppressing the proliferation of lipid-laden foam cells and cholesterol deposits in aortic atherosclerotic plaques in rabbits.
Figure 3. Histologic section through thoracic aorta stained with hematoxylin-oil red O. **A.** Normal control rabbit. **B.** Typical aortic plaque from a rabbit fed the atherogenic diet alone. **C and D.** Representative aortic plaques from rabbits fed the atherogenic diet and treated simultaneously with poly I:C or ABPP, respectively. Areas of lipid deposition in intimal and subintimal cells appear black. Scales are 100 μm.
Table 2. Morphometric Analysis of Intimal Lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>Lesion Area as % Lesion</th>
<th>Frequency of Lesions &gt;20% of Total Vessel Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td>18 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>Atherogenic diet, no drug</td>
<td>26.0 ± 3.2</td>
<td>65</td>
</tr>
<tr>
<td>Atherogenic diet, poly I C</td>
<td>1.3 ± 3.0*</td>
<td>5*</td>
</tr>
<tr>
<td>Atherogenic diet, ABPP</td>
<td>12.7 ± 3.4*</td>
<td>15*</td>
</tr>
</tbody>
</table>

*p < 0.005 compared with atherogenic diet, no drug. Abbreviations as in Table 1.

Antatherogenic Mechanisms of Interferon Inducers

Suppression of initiation or suppression of atherogenesis? The response to injury hypothesis of atherogenesis (1) suggests that endothelial injury may lead to desquamation, platelet adherence, aggregation and release of platelet constituents followed by proliferation of cells in the arterial intima. The sources of initial injury may include chronic hypercholesterolemia as well as immunologic, hemodynamic, mechanical and chemical factors. The monoclonal hypothesis of atherogenesis (19) maintains that, like benign smooth muscle tumors of the uterus, the proliferating cells of atherosclerotic plaques may derive from monoclonal growth of vascular smooth muscle cells. It is not possible to determine from the present data whether the interferon inducers are acting to suppress the initiation or the progression of the proliferative phase of atherogenesis.

Effect on plasma cholesterol and lipoprotein. The administration of human leukocyte interferon has been reported to have profound effects on the metabolism of plasma lipoproteins in human subjects (20), lowering the plasma level of total cholesterol, very low density lipoprotein cholesterol and apolipoprotein A-I. However, the suppression of aortic atherosclerosis observed in our study does not appear to have been achieved by reduction of the experimentally induced hypercholesterolemia. Hypertension also is a powerful atherogenic factor and interferon and its inducers are known to cause transient hypertension and pyrexia after injection (6,7). Although blood pressure and temperature were not monitored in this study, it is unlikely that a blood pressure-lowering effect is an important contributing mechanism because the injections of the interferon inducers were spaced at weekly intervals. Another possibility is that the drugs interfere with the binding and uptake of cholesterol-rich lipoproteins. However, under conditions of elevated serum cholesterol, the expression of high affinity, low density lipoprotein receptors on the surface of normal endothelial and smooth muscle cells would be suppressed and thus any possible drug interference would be negligible. In addition, unpublished results from our laboratory indicate that interferon has no effect on the uptake of normal or modified low density lipoprotein by monocyte-derived macrophages.

Suppression of intimal cellular proliferation. The data strongly suggest that the mechanism of the present approach to reduce rabbit aortic atherosclerosis is related to suppression of intimal cellular proliferation by endogenous interferon. Clearly, the possibility that suppression of the atherosclerosis observed in this study may be mediated by interferon-independent mechanisms cannot be ruled out at this time. Indeed, ABPP has been shown to enhance natural killer cells, cytotoxic macrophages and antibody production (21), while poly I C has an adjuvant effect on immunologic reactivity (22). Whether this atherosclerosis suppression can be attributed directly to interferon is being investigated in our laboratory by studies employing purified rabbit interferon.

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References