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Research Article

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Aspirin Inhibits Development of Coronary Atherosclerosis in Cynomolgus Monkeys (*Macaca Fascicularis*) Fed an Atherogenic Diet

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ABSTRACT The effect of aspirin in the primary prevention of diet-induced atherogenesis in cynomolgus monkeys was studied. The diet consisted of 2% cholesterol and 10% butter by weight for 24 wk. Six monkeys received only the atherogenic diet and five monkeys received the diet plus aspirin, 81 mg/monkey per day. Aspirin did not affect plasma cholesterol levels or aortic atherosclerosis. Platelet aggregation to arachidonic acid was almost completely suppressed. Aspirin decreased significantly the number of coronary vessels with atherosclerotic involvement, and the number of coronary vessels narrowed by 20% or more. Thus, aspirin appears to exert a protective effect in the primary prevention of diet-induced coronary atherosclerosis in a primate model.

INTRODUCTION

Recently, interest has focused on the effect of aspirin in decreasing platelet aggregation and its possible role in the secondary prevention of recurrent myocardial infarctions and strokes. The results of several clinical studies suggest that aspirin exerts a beneficial effect in these conditions (1-3). The present experimental investigation was undertaken to determine whether aspirin is useful in the primary prevention of atherosclerosis. The experimental model we used was the cynomolgus monkey (*Macaca fascicularis*) which was fed an atherogenic diet containing cholesterol and butter. On the basis of experimental work by others (4), it has been postulated that hypercholesterolemia damages the endothelium, which leads to platelet adherence with resultant stimulation of the growth of intimal smooth muscle cells, which, in turn,

leads to the development and enlargement of atherosclerotic plaques. We tested the hypothesis that interruption of this sequence of events by administration of aspirin, a known antiplatelet aggregating agent, might reduce the extent of experimentally induced coronary atherosclerosis in a subhuman primate model.

METHODS

11, young, adult, male cynomolgus monkeys were divided into two groups. One group of six monkeys served as controls (mean body weight 6.0 ± 0.4 kg, \pm SE) and were fed only the atherogenic diet. The other group of five monkeys (mean body weight 6.1 ± 0.2 kg) ate the identical diet and, in addition, received 81 mg of aspirin daily in the form of an orange-flavored children's aspirin tablet. The atherogenic diet, described by Kramsch and Hollander (5), and the drug were given daily, 7 d a week for 24 wk. It contained 2% cholesterol and 10% butter in a specially prepared monkey meal (Ralston Purina Co. Inc., St. Louis, Mo.).

Before the start of the experiment and at monthly intervals thereafter, body weight, plasma total cholesterol (6), and the percent of platelet aggregation to arachidonic acid were determined. Determination of platelet aggregation was performed according to the method described by Born and Cross (7) using an aggregometer attached to a recorder (Chrono-log 350, Chrono-log Corp., Haverstown, Pa.). Standard methods were used to obtain platelet-rich plasma and platelet-poor plasma. The platelet count (8) in platelet-rich plasma was then adjusted to a final concentration of $3.5-4.0 \times 10^5$ platelets/mm³. 0.4 ml of platelet-rich plasma was transferred to a siliconized cuvette, and 0.1 ml of 300 μ g/ml arachidonic acid (Sigma Chemical Co., St. Louis, Mo.) as the aggregating agent was added. Aggregation studies were recorded during 3-5 min, and the percentage of light transmission was read at 3 min considering 0% as the light transmission of platelet-rich plasma and 100% as light transmission of platelet-poor plasma of the same subject.

Hearts were fixed with neutral buffered formaldehyde by perfusion under physiologic arterial pressure (≈ 100 mm Hg) for 20 min. Otherwise the routine procedures established for our laboratory were carried out (9). In brief, atherosclerosis of the aorta and its main branches was graded subjectively in five individual sectors on a scale of 0 (no lesions)

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to 4+ (the most severe lesion). The aortas were graded after fixation in 10% neutral buffered formaldehyde and gross staining with Sudan IV (General Aniline & Film Corp., New York). The aortic grade represents the mean of the five separate sectors. This form of grading has been used for many years and is carried out by two independent observers. Lesions that display mainly lipid infiltration are graded at the low end of the scale and a greater numerical value is assigned as the lesion develops increasing degrees of elevation and fibrosis. In addition, to obtain a more objective assessment, we quantified atherosclerotic involvement of the aorta by tracing on a plastic sheet the outline of the aorta and the outline of all the identifiable lesions as delineated by the Sudan IV stain. We then calculated the percent involvement by cutting the plastic and weighing the involved and normal areas on an analytical balance. In analyzing both the gross and microscopic lesions, we were careful throughout to treat all the specimens as unknowns with respect to the specific group to which they belonged.

Coronary atherosclerosis was assessed microscopically on 5- μ m-thick sections from 10 blocks of tissue from each heart (9, 10). All vessels > 150 μ m in diameter seen in the 10 blocks were counted. The percent of vessels containing atherosclerotic lesions in each monkey was calculated, and the mean for each group is referred to as involvement. With an eyepiece micrometer to measure the diameter of vessels, we obtained the average narrowing in a monkey by taking the mean of the percent narrowing of those vessels with lesions. The value we report as percent narrowing is the mean for each group. The product of these two numbers divided by 100 is called the coronary index. To guard against potential pitfalls in the interpretation of percent narrowing that could result from vascular constriction or dilatation, we normalized the thickness of each atherosclerotic plaque by determining the ratio between the maximum thickness of the lesion and the thickness of the underlying media.

RESULTS

No significant difference was noted between the two groups of monkeys in any of the base-line values. Body weight did not change significantly in either group throughout the study. Aspirin significantly reduced platelet aggregation (Table I). Cholesterol

levels rose significantly ($P < 0.001$) on the atherogenic diet, but significant differences between the two groups did not occur (Table I). The degree of aortic atherosclerosis was not significantly different between the two groups by either method of assessment (Table I).

However, the effects of aspirin on the extent of coronary atherosclerosis were significant. Coronary involvement was lower in the monkeys that received aspirin (controls, $13.8 \pm 1.6\%$ vs. aspirin, $4.9 \pm 1.6\%$, $P < 0.005$ by the Student t test) (Table I). To look at the question of coronary involvement in the vessels at risk in a slightly different way, we compared by chi-square analysis the total number of vessels in each group of monkeys that were free of lesions with the number of vessels that had some atherosclerotic lesion. In the control group, 62 vessels had lesions and 393 vessels were free of lesions; in the group receiving aspirin, 18 vessels had lesions and 357 vessels were free of lesions. This apparent beneficial action of aspirin was statistically significant ($P < 0.001$).

The average extent of narrowing of the lesions was approximately twice as great in the control monkeys, but because of the large SE this difference does not reach statistical significance (Table I). However, if the narrowing of individual lesions is taken into account, it becomes evident that in the control monkeys 35 of 62 (56.4%) plaques produced narrowing of >20%, whereas in the monkeys that received aspirin only 5 of 18 (27.7%) plaques produced narrowing >20% (Fig. 1). Moreover, all 6 control monkeys had vessels with narrowing >20%, whereas in the group treated with aspirin only 1 of 5 (20%) had lesions of this magnitude ($P < 0.05$ by chi-square analysis). Another way of assessing the severity of the atherosclerotic lesions is to compare the total number of vessels at risk. Doing this analysis, we find that 35 of 455 vessels in the control monkeys had

TABLE I
Effects of Aspirin on Arachidonic Acid-Induced Platelet Aggregation, Plasma Total Cholesterol, and Aortic and Coronary Atherosclerosis in Cholesterol and Butter-Fed Cynomolgus Monkeys (*Macaca Fascicularis*)

	Platelet aggregation		Total plasma cholesterol							
	Base line	Mean	Base line	Mean	Aorta		Coronary arteries			
	Control	24-wk	Control	24-wk	Grade	Inv	Inv	Nar	CI	
	%		mg/dl		%		%	%		lesion/ media
Control (n = 6)	66±13	52±7	129±5	703±66	1.8±0.3	49.6±8.0	13.8±1.6	31.1±4.8	4.3±0.9	2.7±0.5
Aspirin (n = 5)	68±17	4±1	133±18	569±55	1.3±0.1	50.3±5.9	4.9±1.6	15.2±6.3	1.1±0.8	1.2±0.2
P	NS	<0.001	NS	NS	NS	NS	<0.005	NS	<0.025	<0.05

Values are mean \pm SE. Inv, involvement; Nar, narrowing; CI, coronary index; lesion/media, ratio of maximum thickness of atherosclerotic lesion to thickness of underlying media.

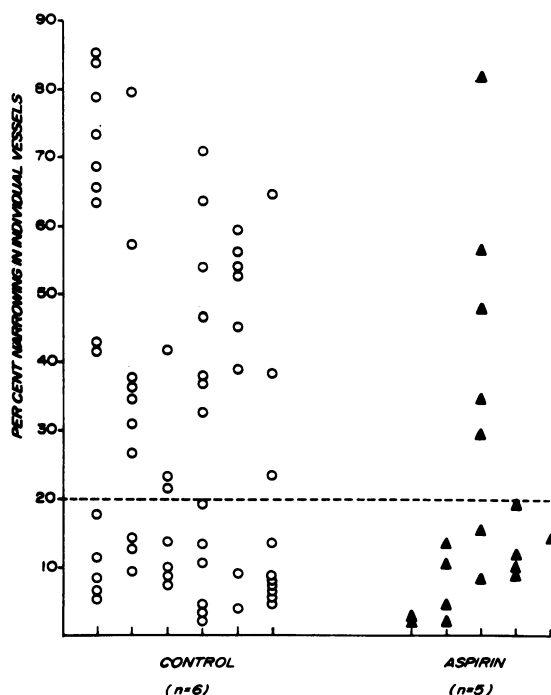


FIGURE 1 Graphic representation of the severity of individual atherosclerotic lesions as observed in each control monkey and each aspirin-treated monkey.

narrowing of 20% or greater, whereas this degree of narrowing was present in only 5 of 375 vessels in the monkeys treated with aspirin ($P < 0.001$ by chi-square analysis).

Furthermore, the ratio of the thickness of the atherosclerotic lesion to the thickness of the underlying media showed a significant difference between the control monkeys and the monkeys treated with aspirin (controls, 2.7 ± 0.5 vs. aspirin, 1.2 ± 0.2 ; $P < 0.05$ by the Student t test) (Table I). When we selected a cut-off point of 2.0, we found that 31 of 62 lesions in the control monkeys exceeded this value, whereas only 2 monkeys treated with aspirin showed a ratio >2.0 ($P < 0.01$ by chi-square analysis).

The coronary index, which takes into account the effects of incidence of involvement and the degree of narrowing, was reduced significantly in the monkeys treated with aspirin (Table I).

Morphologically the lesions were predominantly atheroma with abundant foam cells. In the control monkeys, a number of the lesions showed fibrous caps, as well as necrotic cores and fibrosis within the lesions (Fig. 2). Medial involvement was only rarely observed. The majority of lesions in the monkeys treated with aspirin were predominantly small, pure foam-cellular atheroma. The single more severely affected monkey showed a mixed lesion similar to the less involved controls.

DISCUSSION

The process of atherosclerosis is characterized by lipid deposition and intimal thickening which is predominantly a result of smooth muscle cell proliferation. Smooth muscle cell proliferation has been shown by tissue culture techniques to be enhanced by hyperlipemia (4, 11). It has also been shown that smooth muscle cell growth is stimulated by platelets (4). Therefore, the process of atherosclerosis undoubtedly involves the arterial wall and elements in the blood. Under normal conditions the intact endothelium prevents adherence of platelets. Many substances, however, both endogenous and exogenous, can damage the endothelium. One of these is hyperlipemia, particularly hyperbetalipoproteinemia (12). Once the endothelium is damaged, platelets will adhere to the vascular wall and they will release intracellular components that will lead to further platelet aggregation. Although the exact cellular mechanisms involved are not completely understood at this time, such aggregation contributes to the growth of the smooth muscle cells and to the accumulation of lipids and the growth of the plaque. Aspirin has been shown to prevent platelet aggregation by affecting prostaglandin biosynthesis. By so doing, aspirin can reasonably be expected to be of value in secondary prevention of thrombotic complications in already diseased arteries with damaged endothelium. Several clinical studies suggesting a possible beneficial role in recurrent myocardial infarction and strokes have appeared in the literature (1-3, 13). However, primary prevention of atherosclerosis has to be studied at present in animals.

In the present study with the non-human primate *Macaca fascicularis*, we observed no significant effect of aspirin on plasma cholesterol or aortic atherosclerosis. However, significant protection was produced against coronary atherosclerosis, as manifested by the number of vessels involved in the atherosclerotic process, as well as by the number of lesions with luminal narrowing of $>20\%$. Hollander et al. have investigated the effect of aspirin and other anti-inflammatory agents on the development of aortic atherosclerosis in rabbits (14). In their studies, aspirin did not decrease the size of the aortic atherosclerotic plaques as compared with the controls. However, it changed the morphology of the lesions by reducing the growth of connective tissue elements. The dosage of aspirin in their experiments was three to four times higher per kilogram body weight than the dose used by us. Coronary atherosclerosis was not studied. It should be noted that we also did not observe a beneficial effect on the aortic lesion.

Our results suggest the possible usefulness of aspirin for primary prevention of coronary atherosclerosis produced by an atherogenic diet. Whether

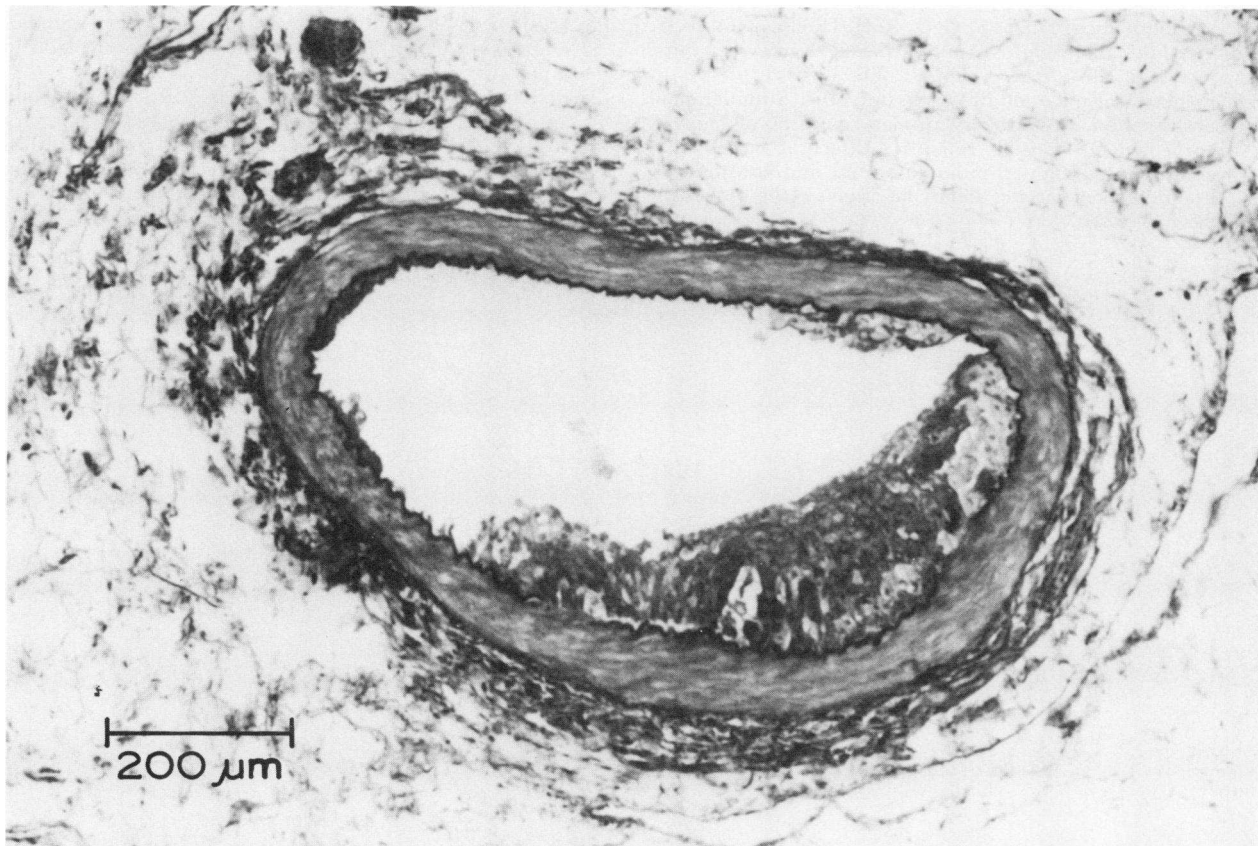


FIGURE 2 Histologic section of a typical lesion from a control monkey fed the cholesterol and butter diet for 6 mo. Gomori's trichrome aldehyde fuchsin stain: elastic tissue is black; collagen and fibrous connective tissue are shades of gray; light areas are foam cell accumulation. Note several breaks in the internal elastic lamella.

the salutary action of aspirin is mediated principally by its antiplatelet aggregating effect or by some other mechanism needs to be elucidated.

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