Atherosclerotic lesions tend to form in areas of the vascular tree subjected to injury—usually mechanical injury. Since injury enhances the phagocytic activity of endothelial cells, the possibility arises that localization of lipid material may be due in part to phagocytosis of serum lipoproteins by injury-activated aortic endothelial cells. Supporting this possibility is the observation that endothelial cells overlying foam cell plaques formed in cholesterol-fed rabbits display phagocytic activity toward intravenously injected colloidal thorium dioxide. In addition, electron microscope studies of these endothelial cells are interpreted as indicating that they are actively removing lipoproteins from the blood by pinocytosis, a process believed to be basically similar to that of phagocytosis. Phagocytosis and/or pinocytosis are further implicated in atherosclerosis in that the foam cell plaques of cholesterol-induced rabbit atherosclerosis, similar in appearance to the fatty streaks or flecks generally considered to be the first gross manifestation of human atherosclerosis, seem to be produced by an aggregation of fat-filled cells (presumably smooth muscles and/or macrophages) that have taken up lipid material deposited in the subendothelial space.

Thus, both the initial transport of lipid to the subendothelial space and its retention there may be dependent in part on phagocytosis and/or pinocytosis. Hence, inhibition of this property of the endothelial cells and of cells in the subendothelial space might result in a decreased rate of progression of atherosclerosis. Antihistaminics have been shown to inhibit the phagocytic activity of tissue macrophages as well as that of injury-activated endothelial cells. In the present work, the possibility that the antihistaminic, chlorpheniramine (Chlor-Trimeton, Schering Corporation), could inhibit atherosclerosis in cholesterol-fed rabbits was evaluated.

**Methods**

New Zealand white male rabbits, weighing initially generally 2,200 to 2,500 Gm., were employed. They were kept on screens in individual cages, given water ad libitum, and each day 100 Gm. of food. The diet was prepared from Purina Rabbit Chow and contained 1 per cent weight (w.) of cholesterol and 5 per cent w. of vegetable oil (Wesson oil). The treated rabbits were given either 2, 4, or 8 mg. chlorpheniramine/day/rabbit (ca. 0.8, 1.6, or 3.2 mg./Kg. body weight/day) in their food. This was done by powdering tablets of chlorpheniramine, followed by mixing a little cottonseed oil (an equal amount of cottonseed oil was also added to the food given to the controls) and then shaking with the base diet to get an even distribution of the chemical. The animals were generally weighed once a week.

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The first work utilized 8 mg./day/rabbit of chlorpheniramine and consisted of three separate parts: the first involved 10 rabbits (5 controls and 5 treated) and was conducted for five weeks, while the second and third lasted for six weeks and involved 16 and 39 rabbits, respectively.

The evaluation of 2 and 4 mg./day/rabbit of chlorpheniramine was done at the same time using 18 controls and a like number of rabbits for each of the treated groups; the animals were fed for five weeks.

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*The rabbits were obtained from the Gopher State Caviary of St. Paul, Minnesota.*
Results and Discussion

In Table 1 are presented, with their standard deviations, the initial and final weights, the weight gains during the experiments, the atherosclerotic grading of the arch and thoracic portions of the aortas, and the total serum cholesterol* at the end of the experiments. Serum cholesterol was determined by a modified Zak method on an aliquot obtained by extracting serum with a 1:1 mixture of acetone and methyl alcohol. The average initial weights, weights gained during the experiments, and serum cholesterols at the conclusion of the experiments were essentially the same for the controls and chlorpheniramine-treated animals.

The ratio of the average degree of atherosclerosis in the aortic arch of the controls to that of the animals treated with chlorpheniramine (8 mg./day/rabbit; ca. 3.2 mg./Kg. body weight) was 1.60/0.4 = 4.0 in the first experiment, 1.37/0.59 = 2.3 in the second, and 1.17/0.675 = 1.7 in the third. Corresponding figures for the thoracic aortas were 0.55/0.05 = 11, 0.5/0.4 = 1.25, and 0.70/0.29 = 2.4, respectively.

Similar results were obtained with the lower dosages of chlorpheniramine. The ratios of the average degree of atherosclerosis in the aortic arch of the controls to that of the groups given 4 mg. chlorpheniramine/day/rabbit (ca. 1.6 mg./Kg. body weight/day) and 2 mg. chlorpheniramine/day/rabbit were 1.26/0.82 = 1.54 and 1.26/0.61 = 2.30, respectively. Corresponding figures for the thoracic aortas were 0.48/0.25 = 1.92 and 0.48/0.13 = 3.70. Thus, chlorpheniramine treatment at all three dosage levels affected a marked and approximately equal decrease in the rate of progression of the cholesterol-induced gross aortic lesions in the rabbit; the data are significant at a level of $P < 0.05$.

The lowest dose of chlorpheniramine employed in these experiments, 2 mg./day/rabbit (ca. 0.8 mg./day/Kg. body weight) is about three times the maximum suggested human therapeutic dose of this compound when employed as an antihistaminic (one 12-mg. repeatable twice a day = 0.34 mg./Kg. body weight/day for a 70-Kg. man). At none of the dose levels employed was there any evidence that the chlorpheniramine had a deleterious effect on the rabbits, judging from their weight gains or behavior.

Although chlorpheniramine reduced the rate of progression of cholesterol-induced rabbit atherosclerosis, it does not necessarily follow that this effect is due exclusively, if at all, to the postulated inhibition of lipid uptake by aortic subendothelial and endothelial cells. For example, it might be due to a decrease in the negative charge of serum lipoproteins secondary to incorporation of the fat-soluble, positively charged antihistaminic; such a change might in some manner, possibly through an enhanced resistance of lipoproteins to phagocytosis, contribute to the observed inhibition of atherosclerosis by chlorpheniramine.

Two attempts, both unsuccessful, have been made to find direct evidence that chlorpheniramine decreases the rate of movement of serum lipids into the arterial wall. In the first attempt, studies of sudan-red-stained frozen sections of aortas from eight rabbits eating a 1 per cent w. cholesterol diet for a few days with (four rabbits) and without (four rabbits) added chlorpheniramine (4 mg./day/rabbit) showed no differences between the two groups; particular attention was given to the areas subjected to mechanical stress, i.e., around the orifices of blood vessels branching from the aorta. Hence, if chlorpheniramine does inhibit passage of lipid into the aortic wall, it was not of sufficient degree to be observed by this technique.

If phagocytosis and/or pinocytosis by aortic endothelial cells are involved in the transfer of serum constituents into the subendothelial space, then possibly a more sensitive means than the study of frozen sections to detect inhibition of this process by chlorpheniramine would be to determine the rate of passage of a labeled material into the aorta in the presence and absence of the antihistaminic. This

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*Serum from the rabbits on 2 and 4 mg. chlorpheniramine was inadvertently disposed of before total cholesterols were determined.
TABLE 1

Effect of Oral Chlorpheniramine on Cholesterol-induced Atherosclerosis in Rabbits

<table>
<thead>
<tr>
<th>Chlorpheniramine dosage</th>
<th>Group*</th>
<th>Weight (Gm.)</th>
<th>Δ Weight</th>
<th>Atherosclerosis†</th>
<th>Serum cholesterol (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Start</td>
<td>Finish</td>
<td>Arch</td>
<td>Thoracic</td>
</tr>
<tr>
<td>8 mg./day/rabbit (3.5 mg./day/Kg. body weight)</td>
<td>(A) Duration: 5 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls (5)</td>
<td>2,309 ± 231</td>
<td>2,750 ± 133</td>
<td>441 ± 130</td>
<td>1.60 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>Treated (5)</td>
<td>2,335 ± 205</td>
<td>2,825 ± 125</td>
<td>430 ± 87</td>
<td>0.40 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>(B) Duration: 6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls (8)</td>
<td>2,134 ± 282</td>
<td>2,696 ± 291</td>
<td>562 ± 288</td>
<td>0.59 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Treated (8)</td>
<td>2,150 ± 343</td>
<td>2,669 ± 250</td>
<td>550 ± 154</td>
<td>1.37 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>(C) Duration: 6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls (19)</td>
<td>2,188 ± 174</td>
<td>2,684 ± 248</td>
<td>496 ± 159</td>
<td>1.17 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>Treated (20)</td>
<td>2,290 ± 215</td>
<td>2,735 ± 240</td>
<td>436 ± 101</td>
<td>0.675 ± 0.718</td>
</tr>
<tr>
<td>4 mg./day/rabbit</td>
<td>Duration: 5 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls (18)</td>
<td>2,455 ± 242</td>
<td>2,838 ± 304</td>
<td>385 ± 165</td>
<td>1.26 ± 1.35</td>
</tr>
<tr>
<td></td>
<td>Treated (18)</td>
<td>2,481 ± 282</td>
<td>2,927 ± 230</td>
<td>446 ± 76</td>
<td>0.82 ± 0.92</td>
</tr>
<tr>
<td>2 mg./day/rabbit</td>
<td>Duration: 5 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls (18)</td>
<td>2,456 ± 242</td>
<td>2,838 ± 304</td>
<td>385 ± 165</td>
<td>1.26 ± 1.35</td>
</tr>
<tr>
<td></td>
<td>Treated (18)</td>
<td>2,446 ± 258</td>
<td>2,888 ± 324</td>
<td>437 ± 173</td>
<td>0.61 ± 0.58</td>
</tr>
</tbody>
</table>

*The data are the averages for each group.
†Graded on basis of 0 to 4+.
‡Standard deviation of the mean.
HARMAN

has been done on four occasions using groups of 10 rabbits each that had been on a regular diet with (five rabbits) or without (five rabbits) added chlorpheniramine (4 mg./day/rabbit) for two days. Each rabbit was injected intravenously with 100 μc. of human serum albumin labeled with I¹³¹ (RISA, Abbott) and then autopsied after 60 minutes. The aortas were removed and the inner layer stripped, through the media, from the arch and thoracic areas, dried to constant weight, and then counted in a scintillation counter; the serum activity was also determined. No consistent difference could be found between control and chlorpheniramine-treated animals. In agreement with previous work, it was noted that the activity in the arch was greater (two to four times) than in the thoracic areas; a similar result was obtained when triolein, labeled with I¹³¹, was given orally and the aortas removed the following day. The fact that chlorpheniramine does not decrease the overall permeability of the aorta to serum albumin does not preclude the possibility that it may do so in local areas of injury. Since neither of the above two approaches has yielded direct evidence that chlorpheniramine inhibits the postulated reaction of the aorta to injury, an electron microscopic study has been started which may provide the desired information.

When this study with chlorpheniramine was started, the localization of particulate material in an area of vessel injury, a process inhibited by antihistamines, was attributed to an enhanced phagocytic activity of the injured endothelial cells. Recent electron microscope studies suggest that the enhancement of phagocytosis is preceded by a pulling apart of the endothelial cells so that there is a direct communication between the lumen of the vessel and the subendothelial space. Hence, particulate matter of suitable sizes can gain direct access to the subendothelial area where it tends to accumulate, presumably due to a filtering action of the basement membrane. If it is assumed that areas of local aortic injury behave in a similar manner, then in areas of the aorta subjected to mechanical injury, such as at points of branching, both processes, i.e., a pulling apart of endothelial cells and enhanced phagocytosis and/or pinocytosis, might be expected to be operative at all times. On the basis of the foregoing assumptions and taking into account recent electron microscopic investigations of human and experimental atherosclerosis, as well as studies on the uptake of lipid by hepatic cells, the following mechanism is suggested for the development of atherosclerosis:

In areas of vessel injury, which for the most part are initially areas subjected to mechanical stress, the endothelial cells tend to be separated so as to permit free access to the subendothelial space of blood constituents; rates of access of blood components would be expected to be in part determined by their size and by that of the "endothelial pores." The endothelial cells in the area of injury would be likely to display enhanced phagocytic and/or pinocytotic activity; this, as well as the tendency to pull apart, might be on the basis of induced histamine. In the case of serum lipoproteins and chylomicrons, the endothelial cells could transport them to the subendothelial space, in pinocytotic vesicles for example, with or without alteration of the material by the cell. In the subendothelial space, any endothelial cell–transported lipid could become aggregated with serum lipoproteins and chylomicrons that had been filtered off against the internal elastic membrane, after having reached the area through the space between adjacent endothelial cells.

Whether or not such an accumulation of lipid actually occurred in the subendothelial space would be determined by the rate at which lipid was delivered to it, for at low delivery rates the lipid material might diffuse into and through the aortic media without any significant increase in the lipid composition of the subendothelial space over that of the plasma, while at high plasma concentration of lipid considerable amounts of lipid might accumulate in the subendothelial area. In the event that lipid does accumulate in
Atherosclerosis and Antihistamine

The subendothelial space then, by analogy with the liver parenchymal cells, cells in this area, such as smooth muscle cells and macrophages, might take up lipid by phagocytosis and/or pinocytosis at a rate faster than they could metabolize the material and thus give rise to foam cells. At the same time, lipid may accumulate in the endothelial cells by a similar process. With the passage of time, the continually accumulating lipid could contribute to further accumulation and extension of the process by a variety of means. For example, the deposition of lipid in the subendothelial space might be expected to be paralleled by an accumulation of the postulated oxidation-polymerized products of serum lipoprotein—products that would be expected to be tissue irritants.

The types and relative amounts of lipid deposited in the subendothelial space would be expected to play a role, for some serum lipid constituents, such as cholesterol, have a marked inflammatory effect on tissue (cholesterol, at least in hepatic cells, appears to interfere with cellular metabolism by disrupting phospholipid synthesis—basis for persistence of foam cells?). Hydrolysis of fatty acid esters in the subendothelial deposits could give rise to insoluble calcium salts, as well as cholesterol, that likewise would be expected to act as irritants. In addition, necrosis of cells in the area could contribute to tissue injury.

By such means as the foregoing, an initial localization of small amounts of lipid could induce changes—further deposition of lipid, enhancement of fibroplastic activity (a process that may in itself tend to further localize lipid in the aortic wall) with resultant fibrosis, and calcium salt deposition—leading to the gross stages of atherosclerosis. This relatively simple view of the pathogenesis of atherosclerosis is in keeping with the known facts and has served as a useful working hypothesis. It was essentially this hypothesis that led to the selection of an antihistaminic, chlorpheniramine, for study as a possible atherosclerosis-inhibiting agent and underlies the belief that the atherosclerosis-inhibiting effect of chlorpheniramine is due to inhibition of aortic reaction to injury.

Summary

Cholesterol-induced rabbit atherosclerosis was inhibited by the antihistaminic, chlorpheniramine, given orally at a rate of either 2, 4, or 8 mg./day/rabbit. Two attempts, both unsuccessful, were made to demonstrate directly the expected chlorpheniramine-mediated decrease in the permeability of the rabbit aortic wall to serum constituents: (1) study of sudan-red-stained frozen sections of aortas from rabbits fed a high-cholesterol diet for a few days, with and without added chlorpheniramine, and (2) a study of the effect of chlorpheniramine on the permeability of the inner aspect of the aorta to 113I-labeled serum albumin. A possible mechanism, based on aortic reaction to injury, is presented to account for the localization and development of atherosclerosis. This hypothesis led to the selection of chlorpheniramine for study as a possible atherosclerosis-inhibiting agent and underlies the belief that the atherosclerosis-inhibiting effect of this compound is due to inhibition of aortic reaction to injury.

References


Atherosclerosis: INHIBITING EFFECT OF AN ANTIHISTAMINIC DRUG, CHLORPHENIRAMINE
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