Lipid-Binding Capacity of Intimal Globulins in Human Aortic Atherosclerosis

By K. S. Mathur, M.D., R. D. Sharma, M.Sc., S. K. Kashyap, M.D., and R. P. Sapru, M.D.

The extent and severity of atherosclerosis of the aorta shows a highly significant correlation with the total cholesterol content of its wall but not with the serum cholesterol. This has suggested the possibility of some factors in the vessel wall that may affect the deposition of lipids in the intima. We have studied the lipid- and the cholesterol-binding capacity of intimal globulins in relation to the degree of aortic atherosclerosis.

Material and Methods

Aortas were obtained from 147 consecutive medicolegal autopsies performed at Sarojini Naidu Medical College, Agra (India). These included 103 male and 44 female subjects, ranging in age from 11 to 80 years (table 1).

The degree of atherosclerosis was studied in each aorta both before and after staining with Sudan IV. The atherosclerotic index was calculated in each case by the methods of Gore and Tejada. On the basis of the atherosclerotic index and the extent and types of lesions, aortas were classified into three arbitrary groups: Group I. Mild atherosclerosis, aortas with less than 50 per cent involvement of the intimal surface, with fatty streaks as the predominant lesions (atherosclerotic index less than 1.0). Group II. Moderate atherosclerosis, aortas showing both fatty streaks and fibrous plaques as predominant lesions, but devoid of ulcerated and calcified plaques (atherosclerotic index 1.0 to 5.0). Group III. Severe atherosclerosis, aortas with extensive involvement of the intimal surface and evidence of complicated and calcified lesions (atherosclerotic index more than 5.0).

To study the lipid-binding capacity of intimal globulins a portion of the intima was removed from the arch of the aorta prior to staining and was soaked between two layers of blotting paper. One gram of intima was weighed and an intimal extract was prepared in a known quantity of normal saline. This extract was subjected to paper electrophoresis with Veronal buffer at pH 8.6. The strips were stained with bromphenol blue for protein and with Sudan black for lipids. The cholesterol, total lipids, and lipid-containing globulins (alpha 1, alpha 2, and beta) were estimated by chemical analysis. For comparison, their values for 1 Gm. of intima were calculated. From these figures the amount of cholesterol and total lipid bound to 1 Gm. of globulins was determined. Then the intimal extract was treated with ether to separate lipids from globulins. The cholesterol and lipid remaining in the extract were re-estimated. These lipids were then mixed with serial dilutions of the original aqueous solution that contains globulins. The combined mixtures were incubated for 20 hours at 37°C, centrifuged, and the uncombined lipids discharged. The solution was subjected to electrophoresis as the intimal extract, and subsequently on chemical analysis recombined lipids and cholesterol with globulins were estimated. Estimates done in our laboratory revealed no appreciable changes in the globulins during this process. Thus lipid- and cholesterol-binding capacity of intimal globulins was determined in each case.

The statistical significance of the results was determined by comparing aortas of group I to group II and of group II to group III.

Observations

In table 2 is given the classification of cases on the basis of the extent and severity of atherosclerosis.

Of the total of 147 aortas, 98 (66 per cent)
Table 2
Classification of Cases on the Basis of Extent and Severity of Lesions

<table>
<thead>
<tr>
<th>Degree of atherosclerosis</th>
<th>Cases</th>
<th>Mean age (years)</th>
<th>Mean atherosclerotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>98</td>
<td>29</td>
<td>0.376 ± 0.682*</td>
</tr>
<tr>
<td>Moderate</td>
<td>31</td>
<td>48</td>
<td>1.86 ± 1.03</td>
</tr>
<tr>
<td>Severe</td>
<td>18</td>
<td>61</td>
<td>13.998 ± 5.60</td>
</tr>
</tbody>
</table>

* Standard deviation.

Table 3
Lipid and Cholesterol Content of One Gram of Intima in Various Groups

<table>
<thead>
<tr>
<th>Degree of atherosclerosis</th>
<th>Mean atherosclerotic index</th>
<th>Lipid content of 1 Gm. of intima (mg.)</th>
<th>Cholesterol content of 1 Gm. of intima (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>0.376</td>
<td>5.76 ± 2.85*</td>
<td>2.28 ± 3.03*</td>
</tr>
<tr>
<td>Moderate</td>
<td>1.86</td>
<td>8.23 ± 4.16</td>
<td>3.11 ± 1.88</td>
</tr>
<tr>
<td>Severe</td>
<td>13.998</td>
<td>9.83 ± 1.72</td>
<td>3.88 ± 1.63</td>
</tr>
</tbody>
</table>

* Standard deviation.

Table 4
Globulins of Intima and the Lipids and Cholesterol Combined with Them in Various Groups

<table>
<thead>
<tr>
<th>Degree of atherosclerosis</th>
<th>Globulin content of 1 Gm. of intima (mg.)</th>
<th>Lipids combined with 1 Gm. of globulins (mg.)</th>
<th>Cholesterol combined with 1 Gm. of globulins (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>18.3 ± 4.71*</td>
<td>367 ± 32.2*</td>
<td>130 ± 69.1*</td>
</tr>
<tr>
<td>Moderate</td>
<td>21.1 ± 6.35</td>
<td>460 ± 36.4</td>
<td>144.7 ± 48.1</td>
</tr>
<tr>
<td>Severe</td>
<td>16.9 ± 3.11</td>
<td>574 ± 85.0</td>
<td>248.2 ± 58.9</td>
</tr>
</tbody>
</table>

* Standard deviation.

Table 5
Lipid-Binding and Cholesterol-Binding Capacity of Intimal Globulins in Various Groups

<table>
<thead>
<tr>
<th>Degree of atherosclerosis</th>
<th>Lipid-binding capacity (mg.)</th>
<th>Cholesterol-binding capacity (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>268 ± 12.06*</td>
<td>111 ± 39.1*</td>
</tr>
<tr>
<td>Moderate</td>
<td>322 ± 38.7</td>
<td>141 ± 32.3</td>
</tr>
<tr>
<td>Severe</td>
<td>468.3 ± 100.6</td>
<td>188 ± 86.6</td>
</tr>
</tbody>
</table>

* Standard deviation.

Table 6
Relationship of Lipid-binding and Cholesterol-binding Capacity with the Degree of Atherosclerosis

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Lip-binding capacity</th>
<th>Cholesterol-binding capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild vs. moderate, t</td>
<td>14.8</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate vs. severe, t</td>
<td>4.1</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
showed mild atherosclerosis, 31 (21 per cent) severe, 22 (13 per cent) moderate, and 18 (13 per cent) severe atherosclerosis. The severity of atherosclerosis increased with age. The mean age was 29 years in the group with mild atherosclerosis, 48 years in the group with moderate, and 61 years in the group with severe.

In table 3 is shown the average cholesterol content of 1 Gm. of intima in different groups. The total lipid and cholesterol content of intima increased with the extent and severity of atherosclerosis from mild to moderate and severe to moderate to severe groups.

In table 4 is given the globulin content of 1 Gm. of intima and the amount of lipids and cholesterol combined with 1 Gm. of globulin in each group. The globulin content of intima showed no appreciable differences with the degree of atherosclerosis. It was 18.3 mg. in the mild and slightly more, 21.1 mg., in the moderate group. The globulin content was lowest in the severe group, 16.9 mg. The amounts of lipids and cholesterol combined with globulins increased with the degree of atherosclerosis from mild to moderate and moderate to severe groups.

In table 5 are given the lipid-binding and the cholesterol-binding capacity of intimal globulins in various groups. The lipid-binding capacity of intimal globulins increased with the degree of atherosclerosis. It was 268 mg. in the mild, 332 mg. in the moderate, and 468 mg. in the severe group. A similar pattern was noticed in the cholesterol-binding capacity—111 mg. in the mild, 141 mg. in the moderate, and 188 mg. in the severe group. Statistical evaluation of the results (table 6) showed a highly significant relation between the lipid-binding capacity and the cholesterol-binding capacity of intimal globulins and the degree of atherosclerosis.

Comment

In the human biological system cholesterol exists in a colloidal phase. The cholesterol-containing aggregates in the intima are supposed to be a complex structure in respect to size, configuration, and composition, the essential constituents being plasma lipids and alpha and beta globulins.

The results of our present study showed that the amount of globulins present in aortic intima was in no way related to the degree of atherosclerosis. There existed, however, a direct relationship between the lipid-binding capacity and the cholesterol-binding capacity of these globulins present in the intimal ground substance with the severity of atherosclerosis. Similar observations were made by Kayahan. In atherosclerosis, there appears more of a qualitative change in the intimal globulins, which may be physical or chemical in nature. This qualitative alteration in the protein moiety of cholesterol aggregates was also suggested by Katz and Stamler. Such changes in the plasma had been shown on electrophoresis by Barr et al., and on ultracentrifugation by Gofman et al. Kayahan, however, has suggested that the lipid-binding capacity of both the serum and the intimal globulins determines the lipid accumulation in atheromatous plaques. Our views based on present findings are in corollary to his, that the metabolic changes of the lipid-binding globulins may be of some primary importance in human atherogenesis.

Summary

One hundred forty-seven aortas obtained from medicolegal autopsies were studied. According to the extent of involvement of the intimal surface and the types of lesions, aortic atherosclerosis was classified as mild, moderate, and severe. The globulin content of the intima and its lipid-binding, and cholesterol-binding capacity were determined in each specimen.

No relation could be established between the globulin content of aortic intima and the degree of atherosclerosis. A direct correlation was observed, however, between the lipid-binding capacity and the cholesterol-binding capacity of the globulins and the severity of atherosclerosis, suggesting the possibility of a qualitative change in the globulins of the ground substance in atherosclerosis.
References


Limitations of the Experimental Method

Experiment is observation made in specifiable and controllable circumstances and it seeks to eliminate that dependence on the personal judgement, the tact, the intuition of the observer which is the weakness of the observational method. If we imagine a problem to be attacked and solved independently by experiment and by observation, the solution by the latter method, in addition to having taken longer and being less exact, will generally have demanded a more arduous and intricate intellectual exercise than the solution by the former.

This economy of experiment in its demands upon certain of the less definable aptitudes of the mind is no doubt a contributory cause of its great success as a method, but probably it has secondary consequences which in the long run and on the large scale are not wholly advantageous. A science which is strictly limited to the experimental method is apt to favour in its workers a tendency to deprecate any great speculative activity and to regard an interest in the free play of ideas for their own sake as evidence of a lack of scientific stability and trustworthiness. Such a distrust of the intellect involves an unsound view of the function of ideas in science. It is a mistake to suppose, as it is so easy to do, that science enjoins upon us the view that any given idea is true or false and there is an end of it; an idea may be neither demonstrably true nor false and yet be useful, interesting, and good exercise. Again, it is poverty rather than fertility of ideas that causes them to be used as a substitute for experiment, to be fought for with prejudice or decried with passion. When ideas are freely current they keep science fresh and living and are in no danger of ceasing to be the nimble and trusty servants of truth. We may perhaps allow ourselves to say that the body of science gets from the steady work of experiment and observation its proteins, its carbohydrates, and—sometimes too profusely—its fats, but that without its due modicum of the vitamin of ideas the whole organism is apt to become stunted and deformed, and above all to lose its resistance to the infection of orthodoxy.—The Collected Papers of Wilfred Trotter, F.R.S. London, Oxford University Press, 1946, p. 120.
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