Localization of Apo-Lipoproteins in Human Carotid Artery Plaques

BY HENRY F. HOFF, PH.D., CAROL L. HEIDEMAN, M.S., GEORGE P. NOON, M.D., AND JOHN S. MEYER, M.D.

Abstract:

Apoproteins from plasma lipoproteins were localized by immunofluorescence techniques in human carotid artery atherosclerotic lesions. These studies were performed in light of the possible importance of these apoproteins in both lipid metabolism and the pathogenesis of atherosclerosis. ApoA-I from high density lipoproteins, apoB from low density lipoproteins, and apoC-III from very low density lipoproteins were localized also as markers for their respective lipoproteins, since the latter cross-react immunologically. The three apoproteins were localized to the same regions of lesions as neutral lipids and, to some extent, fibrinogen. These regions consisted of bands of collagen fibers, usually deeper within the lesion, and the lipid core or atheroma of such advanced lesions. Although the superposition of localization for the three apoproteins and lipid was only 53%, it was suggested that deviation from complete superposition was due to the abrupt changes in lesion structure resulting from the focal nature of the atherosclerotic process. These results suggest that there is a broader specificity than previously implied of the interaction between such lesion components as connective tissue and extracellular lipid accumulations, and apoproteins from plasma lipoproteins. This interaction is believed to result in a net retention within atherosclerotic lesions of human extracranial arteries of these plasma-derived factors, either as free apoproteins or as native lipoproteins.

Additional Key Words

apoA-I, apoB, apoC-III, immunofluorescence techniques, high, low, very low density lipoproteins

Introduction

It is reasonably well established that plasma lipoproteins are linked to the atherosclerotic process in man, although the exact mechanism is still not known. Data from some epidemiological and clinical studies demonstrate a positive correlation between elevations in plasma lipoproteins and extracranial or intracranial atherosclerosis leading to stroke. Recently, data have been presented suggesting that apoproteins, or the protein moiety of these lipoproteins, play a role not only in lipid metabolism but possibly also in the pathogenesis of atherosclerosis. Therefore, the localization of apoA-I from high density lipoproteins (HDL), apoB from low density lipoproteins (LDL) and apoC-III from very low density lipoproteins (VLDL) were determined in human atherosclerotic lesions employing immunofluorescence techniques. The individual apoproteins functioned as markers for their corresponding lipoproteins. Furthermore, the apoproteins were utilized since they do not cross-react immunologically, as do their corresponding lipoproteins. The localization patterns also were compared to those obtained for fibrinogen and albumin, two other plasma proteins.

Methods

ApoA-I, apoB, and apoC-III were isolated from HDL, LDL and VLDL, respectively, by procedures previously reported. Antibodies to these apoproteins were prepared by immunizing goats or rabbits, and globulin fractions of the individual antisera were conjugated to fluorescein isothiocyanate (FITC). The coupled antibodies were further purified by affinity chromatography, employing Sepharose 4B to which the appropriate apoprotein had been coupled. The column of Sepharose-apoprotein complex functioned as a solid-phase immunoadsorbent. FITC-conjugated antibodies to fibrinogen and albumin were obtained commercially (Hyland Products, Costa Mesa, California).

Carotid artery bifurcations with atherosclerotic involvement were obtained at surgery from 20 human subjects. The 20 arterial specimens were further subdivided giving a total of 65 arterial blocks studied. All were classified as advanced atherosclerotic lesions with varying amounts of fibrotic and atheromatous involvement. Cryostat sections (4-μ thick) of each specimen were then incubated for one hour at 23° in the appropriate apoprotein localization medium or stained for neutral lipid with oil red O. An attempt was made to use as close to serial sections from each specimen as technically possible for each series of localizations. The direct immunofluorescence technique was utilized to localize apoB, whereas the indirect technique was used to localize apoA-I and apoC-III. Controls employing non-immune sera were consistently negative. A Leitz Orthoplan photomicroscope equipped with an HBO 200W

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mercury light source, UG-I, primary filter, K 430 secondary filter, and dry dark field condenser was used to localize and document specific fluorescence.

**Results**

The three apoproteins and neutral lipid were localized to similar areas within the carotid artery plaques. These areas consisted of bands of collagen usually deeper within the intimal lesion, and in the lipid core or atheroma of these lesions (fig. 1). The 65 separate lesions studied were classified from 0 to 4 according to the maximum number of apoproteins and lipid localized together in one major area of the plaque. As can be seen in table 1, 34 out of 65, or 53%, of the plaques demonstrated the superimposed localization of all four factors in at least one major area of the lesion. However, the percentage was 88 when tabulating those lesions demonstrating the superimposed localization of three out of four factors. By contrast, 0% of the lesions showed none of the factors, and only 6% showed the presence of only one factor. In general the structure of lesions differed markedly from section to section in those specimens illustrating less than three out of four superimposed localizations. Tabulating the frequency of occurrence in all positive areas within all the lesions studied gave the following results: neutral lipid > apoB > apoA-I = apoC-III. Fibrinogen was found in 20% of all lesions, while albumin was found in only 8%. Twenty-five percent of the areas demonstrating maximum superposition of apoprotein and lipid localizations for each lesion also contained fibrinogen, but none contained albumin.

**Discussion**

The results of this study demonstrate a trend toward complete superposition of the localization patterns of apoproteins from HDL, LDL, VLDL and neutral lipid. Similar superpositions of localizations also were obtained for the coronary artery, cerebral artery and aortic beds. These localization patterns were similar to those obtained previously for LDL or apoB in human lesions. The departure from complete superposition of localization patterns is believed to result from the abrupt structural changes in the arterial lesions from one plane of sectioning to another, due to the focal nature of the atherosclerotic plaques.

**Table 1**

<table>
<thead>
<tr>
<th>No. specimens</th>
<th>Classification*</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
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*Classification is based on the maximum number from 0 to 4 of the factors apoA-I, apoB, apoC-III and neutral lipid with superimposed localizations in at least one major area of each lesion.

![FIGURE 1](http://stroke.ahajournals.org/)

Carotid artery fibrous plaque. Localization of apoB in the lipid core (C) and associated with connective tissue using immunofluorescence techniques. Positive fluorescence is depicted at light areas closely matching the localization of neutral lipid. The localizations of apoA-I and apoC-III were similar to that of apoB, but not as extensive. L = lumen. Magnification × 100.
process. This is consistent with the observations in this study that there was a variability in lesion structure from section to section within the same tissue block. This variability was greatest in those cases showing less than complete superposition of localization patterns. Differences in the observed frequencies of localization of neutral lipid and the three apoproteins may be indicative of the amounts of these factors in the lesion, but they also may be the result of such technical variables as antibody titer or fluorescein to protein ratios of conjugated antibodies.

The presence of apoC proteins in human atherosclerotic lesions has been described previously in a preliminary report. HDL proteins, however, have not been consistently detected in such lesions by immunofluorescence techniques, but were demonstrated by immunochromical procedures in arterial lesion extracts. HDL protein uptake by the rat aorta also has been demonstrated by autoradiographical techniques. Since the HDL, LDL, and VLDL fractions have common antigens, their localization in lesions cannot be determined with antibodies raised against these lipoproteins. Similarly, since anti-apoC-III reacts with both VLDL and HDL, the localization of apoC-III cannot be used as a definitive marker for HDL. However, the simultaneous presence of apoB and apoC-III in lesions strongly suggests the presence of some VLDL or possibly of VLDL "remnant particles" which recently have been suggested to be implicated in the pathogenesis of atherosclerosis.

The superimposed localization of apoproteins and neutral lipid in carotid artery lesions suggests the presence of intact lipoproteins, but more detailed studies characterizing these lipoproteins in arterial extracts are necessary to definitively answer this question. The presence in human arterial lesions of low density lipoproteins has been demonstrated in extraction studies and by immunochromical procedures.

Numerous plaque constituents such as glycosaminoglycans, elastin, collagen and cell debris have been implicated in the retention mechanism of lipoproteins in human atherosclerotic lesions, in particular of the cholesterol-rich LDL. From this present study it appears that certain fibrotic and lipid-rich regions, present only in atherosclerotic lesions, have the ability of interacting with all the apoproteins or native lipoproteins and, to a certain degree, with fibrinogen, resulting in net accumulation of these plasma-derived constituents within the arterial intima. It is still not clear if the interactions involve the apoproteins or lipids associated with these apoproteins. It has been suggested previously that only LDL or apoB and fibrinogen were specifically retained in atherosclerotic lesions. From the present results it would appear that this interaction is not as specific as previously thought, since all the apoproteins that were studied from human plasma lipoproteins are retained in the carotid artery plaques.

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References


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