Apolipoprotein Localization in Human Cranial Arteries, Coronary Arteries, and the Aorta

HENRY F. HOFF, PH.D.

SUMMARY  Apolipoproteins from human plasma high density (HDL), low density (LDL) and very low density lipoproteins (VLDL) were visualized in human arteries employing immunofluorescence techniques. Comparison between the localization patterns in extracranial and intracranial arteries and those in coronary arteries and the aorta was made. ApoA-I from HDL, apoB from LDL, and apoC-III from VLDL, as well as neutral lipid, were all localized to connective tissue and extracellular lipid pools in atherosclerotic lesions, and only to areas of intimal thickening in grossly "uninvolved" arteries.

Some studies in humans have implicated elevated levels of plasma low density (LDL) and very low density (VLDL) lipoproteins as risk factors for atherosclerosis in coronary as well as extracranial and intracranial artery beds. Dietary-controlled studies in experimental animals have corroborated this correlation. We studied localization patterns of LDL, apoB and apoE from other plasma lipoproteins in human arteries. ApoA-I from HDL, apoB from LDL, and apoC-III from VLDL were localized in a number of different arterial beds in both uninvolved and atherosclerotic regions and constituted indicators of lipoprotein accumulation. These results showed that these three apoproteins were localized primarily together with neutral lipid, and specifically to lesion connective tissue and atheromas. Similar results were obtained in a study of carotid artery bifurcations which were excised because of atheromas. In the present report we have used extended previous studies for the purpose of ascertaining whether differences in prevalence of atherosclerosis in various vascular beds could be correlated with differences in lipoprotein accumulation.

Methods

The procedures for isolating apoA-I, apoB, and apoC-III from HDL, LDL, and VLDL, respectively, as well as the immunization procedures for raising specific antibodies were described previously. Globulin fractions of antibodies were first conjugated to fluorescein isothiocyanate (FITC) and then further purified by affinity chromatography on a column of Sepharose to which the appropriate antigen had been covalently coupled. The column thus functioned as a solid phase immunoadsorbent.

Segments from the following arteries were obtained at autopsy from 35 individuals ranging in age from 18 to 78 years: right and left middle cerebral arteries, the basilar artery, origin of the right and left internal carotid arteries; the right and left anterior descending, and left circumflex coronary arteries; and the ascending thoracic and abdominal aortas. These arteries were grouped into the following three categories: extracranial and intracranial arteries, coronary arteries, and the aorta.

The degree of superposition of localizations was similar in each vascular bed, and within the error resulting from the structural changes due to the focal nature of the atherosclerotic process. These results suggest a broad specificity in localization of apolipoproteins in most arterial lesions, and suggest that no differences in apolipoprotein accumulation exist between extracranial and intracranial arteries, coronary arteries, or the aorta. Variations in prevalence for atherosclerosis in each arterial bed must be accounted for on other bases.

Results

As was documented previously, apoA-I, apoB, and apoC-III and neutral lipid were found in the same regions of atherosclerotic arteries. These consisted of lipid cores or atheromas of plaques; in basilar (fig. 1a) or coronary arteries (figs. 1b and c) and along elastic fibers (fig. 1d).

In extracranial and intracranial arteries, 55% of plaques and 58% of fatty streaks demonstrated superposition of all three apoproteins and lipids (table 1). Eighty-nine percent of plaques and 83% of fatty streaks showed at least three out of the four factors together. In contrast, 76% of the uninvolved arteries from this arterial bed did not show presence of any of the four factors, while 96% showed no greater than one.

In coronary arteries, 65% of plaques and 67% of fatty streaks demonstrated the superposition of all four factors (table 1). Ninety percent of plaques and 84% of fatty streaks showed at least three out of the four factors together. Some apoproteins were found in the ten uninvolved arteries studied, always in areas of intimal thickening.

In the aorta, 54% of plaques and 44% of fatty streaks showed superposition of all four factors (table 1). Eighty-three percent of plaques and 88% of fatty streaks demonstrated at least three out of the four factors together.

From the Department of Neurology, the Baylor-Methodist Center for Cerebrovascular Research, Baylor College of Medicine, and the Methodist Hospital, Houston, Texas 77030.

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As in the coronary arteries, some apoproteins were found in the four segments of uninvolved aortas, but always in areas of diffuse intimal thickening.

In extracranial and intracranial arteries—the frequency in the presence of apoproteins in plaques followed the progression: apoB > apoC-III > apoA-I (table 2). In fatty streaks the progression was: apoA-I > apoC-III > apoB. ApoA-I and apoB were found in four uninvolved arteries. Generally, the frequency in superposition of localization of any two apoproteins was the same in both plaques and fatty streaks. The superposition of localization of all three apoproteins likewise was almost identical in plaques and fatty streaks.

In coronary arteries the frequency in appearance of each apoprotein individually was almost identical, while in fatty streaks it frequently followed the progression: apoC-III > apoA-I > apoB. Seven out of the ten uninvolved coronary arteries showed apoB, always in diffuse intimal thickening. The frequency in appearance of any two apoproteins together was around 80% for both coronary plaques and fatty streaks, while the frequency of finding all three apoproteins together was 75% for both lesion types. Four out of ten uninvolved arteries also showed the superimposed localization of all three apoproteins, again in areas of diffuse intimal thickening.

In the aortic plaque the frequency in appearance of apoproteins was: apoB > apoC-III > apoA-I (table 2). In aortic fatty streaks it was: apoA-I > apoB = apoC-III. Two out of the four uninvolved aortas studied showed the presence of each apoprotein. The frequency in superposition of localization of any two apoproteins in aortic plaques followed the progression: apoC-III + apoA-I > apoB + apoC-III > apoB + apoA-I. Fifty-four percent of the aortic plaques and only 30% of aortic fatty streaks showed superimposed localization of all three apoproteins.

Discussion

This study represents an extension of a study reported earlier describing the frequency with which localization patterns of apoA-I from HDL, apoB from LDL, apoC-III from VLDL, and neutral lipid were superimposed.9

Only the atherosclerotic lesions consistently demonstrated

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**FIGURE 1. Localization of apoB (white areas) in human atherosclerotic lesions employing immunofluorescence techniques.**

- **a:** apoB is seen as a granular array in the lipid core (large arrow) of a basilar artery plaque. Some connective tissue also reacts positively (small arrow). The white banded material at the lower part of the micrograph represents nonspecific autofluorescence of connective tissue (X100).
- **b:** apoB is seen in the intimal lipid core (arrow) of a coronary artery advanced lesion. Internal elastic membrane (el) shows autofluorescence, as does the area on the lower left of the micrograph. L = lumen (X100).
- **c:** higher magnification of apoB (arrow) in the lipid core of a coronary lesion. Elastic fibers (el) deeper in artery show autofluorescence (X240).
- **d:** apoB is localized along reduplicated or newly formed elastic fibrils (arrow) only on the lumen side of the autofluorescing internal elastic membrane (el) (X240).
TABLE 1  Frequency of Maximum Superposition of Apoproteins and Lipid in Human Arteries

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*Each arterial specimen was classified from 0 to 4 according to the maximum number of apoproteins (apoA-I, apoB, and apoC-III) and neutral lipid that had superimposed localizations in any major region of the artery.

localization of all three apoproteins studied. Plaque connective tissue and atheromas were the primary regions of apoprotein accumulation. These results are consistent with earlier studies on apoB and apoC-III localization from this laboratory and others, and suggest that there exists an affinity between the apoproteins and these lesion components. It cannot be ruled out that neutral lipid deposited in necrotic zones and along connective tissue fibers is the major material interacting with apoproteins. Conversely, the lipid moiety of the lipoprotein rather than the apoprotein may be reacting with plaque components.

The major aim of this study was to ascertain whether any differences could be found between the localization patterns of apoproteins in extracranial and intracranial arteries and those in coronary arteries and the aorta of humans. Both the frequency of maximum superposition of apoprotein and lipid localizations and the frequency of finding all three apoproteins together in atherosclerotic lesions were higher in the coronary artery bed than in either the extracranial and intracranial arterial bed or the aortic bed. It is conceivable that this result may represent altered affinities for apoproteins by different vascular beds. It appears more likely, however, that both of these differences, as well as the departure within lesions from complete superposition of localization patterns, result from the focal structural changes in the artery due to the atherosclerotic process. It seems quite likely that these focal changes are more abrupt in smaller arteries such as intracranial arteries than in the somewhat larger coronary arteries.

A large fraction of the arteries studied that were grossly classified as uninvolved showed microscopically the presence of lipid in the arterial intima, and were therefore put into the fatty streak category, according to the definition employed in this study. This resulted in an inordinately low number of specimens in the uninvolved artery group, particularly for coronary arteries and the aorta, making statistical evaluation difficult. The results on uninvolved arteries, however, do show that apoprotein accumulation occurs only in areas of intimal thickening. Although extensiveness of positive fluorescence for the three apoproteins was not as great in uninvolved arteries as in lesions, we must await quantitative studies on such arteries to ascertain differences in apoprotein accumulation.

Our results suggest that, aside from geometrical differences in atherosclerotic lesions, there are no major qualitative differences with respect to apoprotein accumulation between the three vascular beds studied. The differences in prevalence of human atherosclerosis between the extracranial and intracranial arterial beds, and the coronary

TABLE 2  Frequency of Apolipoprotein Localization and Superpositions in Human Arteries

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<th>Specimen type</th>
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artrial and aortic beds, do not appear related to accumula-
tion of apoproteins or their respective lipoproteins.

Acknowledgment

The author is indebted to the members of the Pathology Service, the
Methodist Hospital for assistance in procuring arterial specimens, to Dr.
Richard L. Jackson for providing purified apoproteins and their respective
antibodies, and to Carol L. Heideman for providing excellent technical
assistance.

References

1. Kannel WB, Dawber TR, Friedman GD: Risk factors in coronary heart
disease: An evaluation of several serum lipids as predictors of coronary
1964
2. Kannel WB, Gordon T, Dawber TR: Role of lipids in the development of
orary heart disease. 1. Lipid levels in 500 survivors of myocardial infarc-
factor in occlusive cerebral vascular disease (stroke). JAMA 232:
262-266, 1975

SUMMARY A 16-year-old boy, who had sudden left-sided hemi-
plegia, died two weeks following onset of symptoms. A right carotid
angiogram showed stenosis at the termination of the internal carotid
artery. The middle cerebral artery had a beaded appearance and
some of its branches were occluded. A basal "moyamoya" syndrome
and transdural anastomoses were present. At autopsy, multiple intra-
cranial dissecting aneurysms were found. Arteries of the body dis-
played fibromuscular dysplasia (FMD). The relevance of dysplastic
changes of intracranial arteries and the relationship to moyamoya
syndrome are discussed.

Fibromuscular Dysplasia and Multiple Dissecting
Aneurysms of Intracranial Arteries

A Further Cause of Moyamoya Syndrome

P. PILZ, M.D.,* AND H. J. HARTJES, M.D.†

MANY REPORTS have been published in recent years on
fibromuscular dysplasia (FMD) and moyamoya syndrome
and they have been reviewed in the Journal de Neuro-
radiologie, volume 1, numbers 1 and 2, 1974. We present a
case which shows features of both these conditions and, in
addition, dissecting aneurysms of the intracranial arteries.

Case Report

RM, a 16-year-old boy, had had repeated attacks of mid-
dle ear infection and frontal sinusitis in recent years. The
family history was unremarkable. On March 10, 1974, after
throwing a snowball he suddenly had a severe headache and
lost consciousness. After admission to the hospital he
regained consciousness but was unable to move the left side
of his body and he still had a headache. On physical ex-
amination he was alert and correctly orientated but a little
euphoric. He laid on his left side and had no neck stiffness.
He had a left hemiparesis affecting more severely the left

arm and hemianesthesia of the left side. The left plantar
response was extensor and the right was flexor. He had slight
miosis and ptosis on the right side, no visual disturbances,
and normal fundi. No bruit was heard in the neck. The blood
pressure was 110/70 mm Hg, the blood cell count was nor-
mal, and the ESR was 6 mm in the first hour. The cerebro-
spinal fluid was clear and the pressure was 250 mm H2O.
The cell count was 1 cell per cubic millimeter, and Pandy's
test was negative. The EEG showed a marked delta focus in
the right frontoparietal region. X-rays of the skull were nor-
mal. On March 19, 1974, the right common carotid artery
was punctured during general anesthesia. The cervical part
of the internal carotid artery showed regular corrugation
(stationary waves). Stenosis was present in the supra-
clinoidal portion of this artery. The terminal part of the in-
ternal carotid artery and its main intracranial branches
showed multiple, repeated stenoses (beaded appearance) and
some branches were occluded. The frontopolar artery was
extremely thin.

The lenticulostriate arteries and the anterior choroidal
artery were very prominent. A flush was present in the
striae and the contour of the head of the caudate was out-
Apolipoprotein localization in human cranial arteries, coronary arteries, and the aorta.

H F Hoff

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