Intimal thickenings of the human facial artery: an immunohistochemical and morphometrical study

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SUMMARY

In the present study, we undertook an experimental study of the structure, immunohistochemistry and morphometry of the human facial artery. The structure of this artery belongs to the muscular type and we observed pathological intimal thickenings, although no physiological mechanisms of regulation of blood flow (cushions) were located, not even at the emergence of collateral branches.

Our immunohistochemical findings suggest that one of the most important causes of the development of intimal thickenings is the migration of muscular cells from the media to the intima.

The morphometric study confirmed this hypothesis, revealing a more positive reaction to α-actin antibodies in the muscular layer of arteries without intimal thickenings than in thickened arteries.

Key words: Facial artery - immunohistochemistry - morphometry - human.

INTRODUCTION

The latest methods of morphological investigation have made it possible to observe certain special features of the arterial walls. One of these peculiarities is the so-called intimal thickenings.

The American Heart Association (1991) established two types of intimal thickenings:

1. Physiological intimal thickenings, involving an adaptive response of the arterial wall to variations in blood flow and pressure. There are two types: a) eccentric intimal thickenings, also called "pads" or "cushions", which show a focal and irregular distribution (Moffat, 1959; Velican and Velican, 1977; Stary, 1987; Diaz et al., 1987; Whyte et al., 1996) and b) "diffuse intimal thickenings", which occupy the whole of the vascular circumference (Schewenke and Carew, 1988); Spring and Hoff, 1989).

2. Pathological intimal thickenings (atherosclerotic), which are characterized by the intimal storage of lipids and the presence of certain peculiar carbohydrates and types of collagen in the extracellular matrix.

The differences between a physiological thickening and an atherosclerotic one are not always clear and further confusion may arrive since the adaptive areas also tend to undergo atherosclerotic degeneration (Glagov and Zarins, 1989).

Here, we studied the structural, inmunohistochemical and morphometric pattern of the intimal thickenings in the human facial artery.

MATERIALS AND METHODS

We studied 43 human facial arteries obtained from biopsies of the submaxilar portion of these arteries. We chose this part because of its accessibility during radical neck dissections and due to its reduced anatomical variability.

The specimens were transversally sectioned in segments of 2 cm and fixed in 10% formol.

After being dehydrated through graded alcohols and embedded in "paraplast" the tissues were sectioned with a Leitz microtome (5-7μm) and were stained with Martins' trichrome, Mas-

Submitted: November 28, 1997
Accepted: May 15, 1998

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son's trichrome, Alcian blue-P.A.S.-Brilliant yellow, Orcein and Verhoeff methods.

Anti α-actin antibodies were applied for the immunohistochemical study. Tissue fixation for the α-actin reaction was carried out with 2% PBS. Incubation in antibodies was performed with α-actin 1/400 from SIGMA® diluted 1% in 1% PBS+BSA for 1 hour at room temperature, followed by incubation in biotinylated antibodies from AMERSHAM® diluted 1% in 1% PBS+BSA for 30 minutes at room temperature. Development was carried out with DAB. Enhancing Solution of BIOSYS® diluted 10% in DAB. Substrate Horseradish Peroxidase from VECTOR® for 15 minutes at room temperature and in the dark.

For the morphometric analysis the QUANCUOL programme (INSERM 441 Laboratory, Pessac-France) was used. This programme measures the variables through a study of colours. The following parameters were measured:

- Surface of média layer per transverse section (μm^2)
- Surface of intima layer per transverse section (μm^2)
- Percentage of collagen in the arterial wall (media and intima)
- Percentage of positive antigen-anti α-action antibody reaction in the intima (high and low positivity)
- Percentage of positive antigen-anti α-actin antibody reaction in the media (high and low positivity)

The magnification used in the morphometric study was 100x for the general morphology and 200x and 400x for the intimal thickenings.

Values were compared with an "a posteriori" test (q of Newman-Keuls and tables, based on the variance and the standard error); $p \leq 0.05$ was regarded as significant and $p \leq 0.01$ as highly significant.

Results

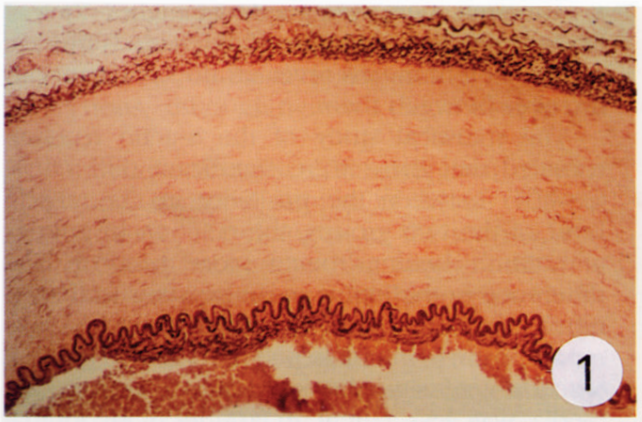
The *structure* of the facial artery belongs to the muscular type. The intimal layer was formed by a row of endothelial cells with their nuclei projecting into the lumen. The media, separated from the intima by an undulating and well-defined internal elastic lamina, was composed of smooth muscle cells arranged transversally and surrounded by collagen and elastic tissue, which was mostly found in the inner half of this layer. No abrupt limits between the media and the adventicia were observed. This latter layer was very thick and rich in vessels and nerves.

In our samples we observed the main stages of development of intimal thickenings. Of 43 arteries, 25 showed intimal thickenings, the average age of the patients being of 64.93 years, with maximum and minimum values of 88 and 41 years respectively.

At the beginning we could see a mere duplication of the internal elastic lamina or a small gap with a widening of the subjacent subintimal space (Figs. 1 and 2). In a first stage the thickening occupied a small "half moon"-like area (eccentric thickening) or included the whole circumference (diffuse thickening). In some cases (Figs. 3 and 4) the lumen was completely occluded. In amplified details of the subintimal space we observed "elastosis", and the morphological foundation of the genesis of an intimal thickening, which, for us, consists of the migration of smooth muscle cells from the media to the intima. Fig. 5, stained with Masson's trichrome, shows muscle cells from the media approaching the internal elastic lamina as though they were going to traverse it;on the other side of the lamellae, in the subintimal space, are muscle cells arranged longitudinally that seem to arise from having traversed the lamina. With the Alcian blue-P.A.S.-Brilliant yellow technique, a blue staining, typical of acid mucosubstances such as chondroitin sulphates, was clear in the intimal thickenings (Fig. 6).

Immunohistochemical assays were performed to clarify the muscle constitution of the intimal thickenings. We stained the areas that expressed α-actin antigens when we applied the corresponding antibodies. The results in arteries without intimal thickenings showed the dominant staining of the media. By contrast, arteries with small thickenings showed some areas stained in the subintimal space (Fig. 7) and when the thickening was morphologically well developed, the reaction at this level (both strong and smooth) was very clear (Fig. 8).

Fig. 1.– Beginning of an intimal thickening: duplication of the internal elastic lamina and widening of the subjacent subintimal space. Orcein. x 100.
Fig. 2.– Detail of the polymorphic internal elastic lamellae: undulating and divided into two coats. Orcein. x 400.
Fig. 3.– Detail of the internal elastic lamina: small gaps through which muscle cells from the media pass to the subintimal space. Note the presence of muscle cells in this space as well as the high content of collagen. Martins' trichrome. x 400.
Fig. 4.– Note the presence of a large intimal a thickening occluding part of the arterial lumen. Orcein. x 100.
Fig. 5.– Detail of an intimal thickening. Note the presence of nuclei of smooth muscle cells (right). Masson's trichrome. x 400.
Fig. 6.– Note the definition of the internal elastic layer constituted by hyaluronic acid. The intima is rich in highly sulphated mucosubstances. Alcian blue- P.A.S.-Brilliant yellow. x 400.
Fig. 7.– Detail of the beginning of an intimal thickening. Positive reaction in the subendothelial space although in the media it is more important. Anti α-actin antibodies. x 600.
Fig. 8.– Detail of a well-developed intimal thickening. Note the intense reaction (smooth and strong) in the subintimal space (right). Anti α-actin antibodies. x 600.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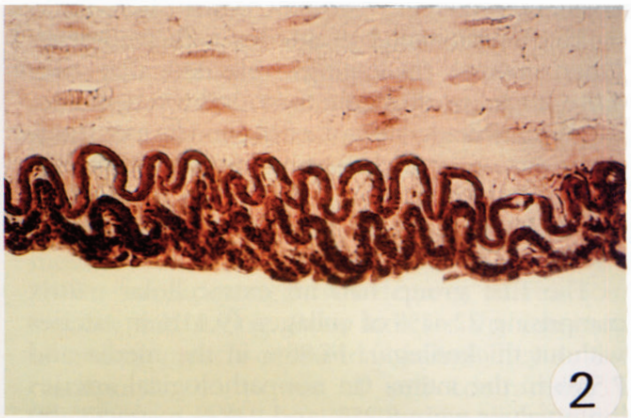
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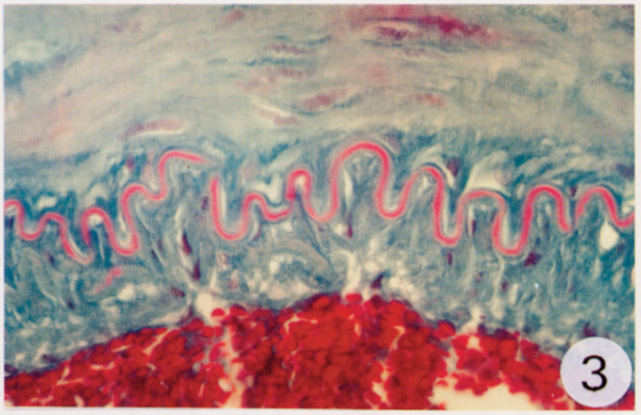
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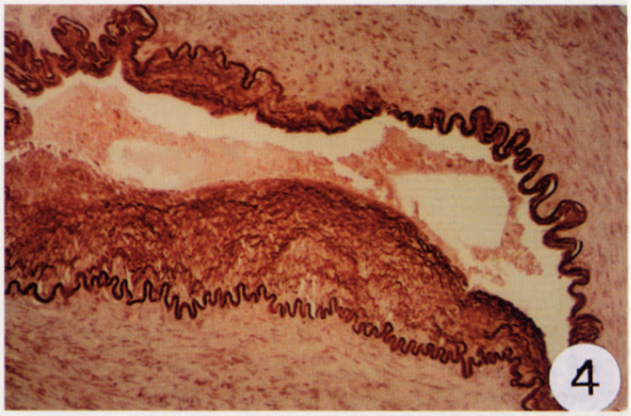
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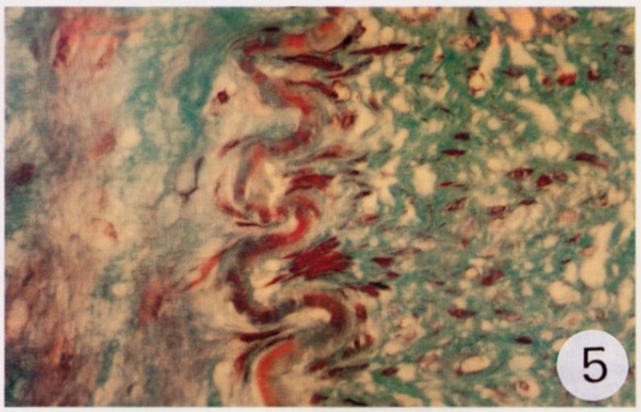
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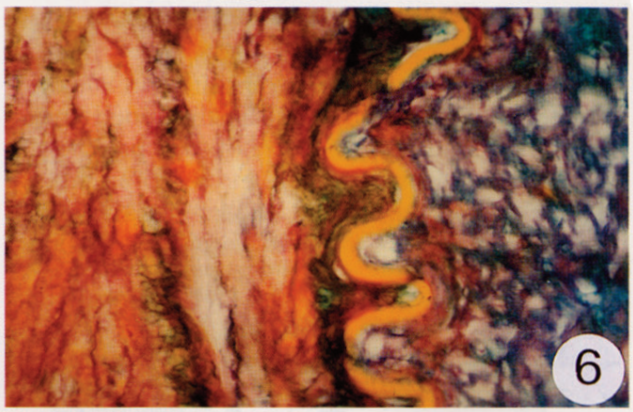
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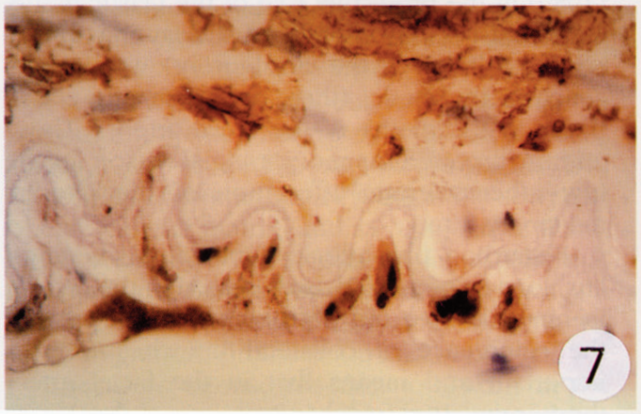
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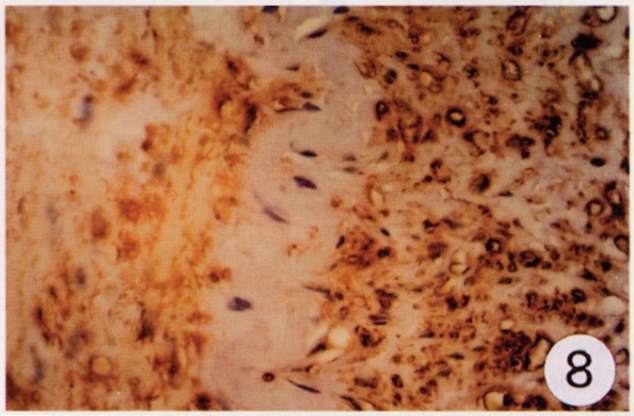
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It should be stressed that we did not observe physiological intimal thickenings in any of the arteries studied, not even at the emergence of collateral branches.

The *morphometry* of arteries with well-developed intimal thickenings revealed that they were larger in diameter than unthickened arteries. The average surface of the media per transverse section of thickened arteries was 1,394,462 μm^2 and the intima 208,041 μm^2. In arteries without thickenings these values were 164,484 μm^2 and 1,445 μm^2 respectively.

The first group had an extracellular matrix comprising 22.41% of collagen (9.11% in arteries without thickenings): 14.86% in the media and 7.55% in the intima (in nonpathological arteries these values were 8.25% and 0.86% respectively) (Table 1).

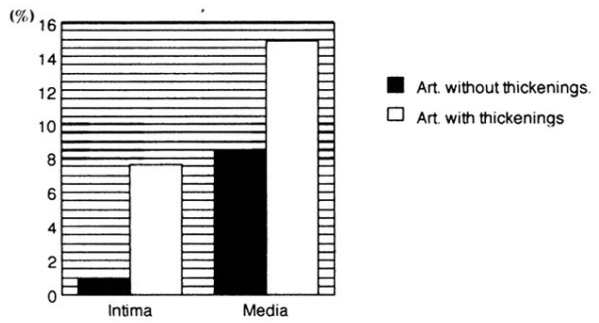

Table 1.– Arithmetic means of percentages of collagen in the two coats (intima and media) of arterial walls.

The α-actin reactions were positive in 80.06% of the whole area of the wall in thickened arteries. In the media from 53.95% of total positivity, 14.36% was a strong reaction and 39.65% a smooth staining. In the intimal thickenings the average reactions were high: total 26.11%, strong 7.73% (Table 2).

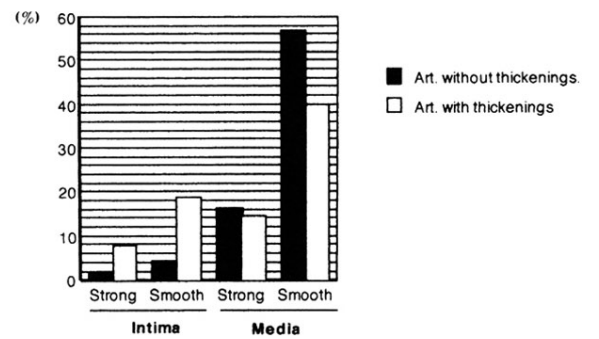

Table 2.– Arithmetic means of percentages of strong and smooth antigen-anti α-actin antibodies reactions in the intima and the media of facial arteries.

Comparison of the parameters disclosed differences between the two groups. Statistically highly significant differences were found in the size and the lumen between arteries with and without thickenings: facial arteries with a media of more than 1,300,000 μm^2 had a higher risk of developing intimal thickenings than arteries with a surface of the muscle layer of less than 200,000 μm^2.

Although there was a 1.8-fold higher content of collagen in the media of thickened arteries, comparison of means revealed no significant differences between them.

The statistical study of the α-actin reaction revealed that there were significant differences ($p<0.05$) between the positivity in the muscle layer of the two groups, the reaction in arteries without thickenings being more positive. The difference in the intima was also statistically significant ($p<0.01$) but in this case the reaction was more positive in thickened arteries. Regarding reaction intensity, the smooth reaction in the media was less patent in arteries with intimal pathology than in normal arteries ($p<0.05$). The strong reaction did not show significant differences. The difference of smooth reaction in the intima was significant ($p<0.05$), the percentage of thickened arteries being higher and the same was the case with the strong reaction ($p<0.01$).

Discussion

Our study confirms that the facial artery is not free of suffering atherosclerotic degeneration This phenomenon is age-related and we even saw one case of complete occlusion of the lumen. In our view this has two implications: the first is that the lack of ischemic problems and the success of reconstructive techniques based on this artery are not due to the absence of intimal pathology, but rather to the large number of arterioarterial anastomoses and also because this artery has no terminal branches, unlike others such as the coronary arteries. The second conclusion is the correlation in this artery between the lack of elastic fibres and the greater tendency to develop atherosclerosis. On this sense we agree with Díaz et al. (1987) and Sims et al. (1989) in the protective role of the elastic tissue, as a well-defined internal elastic lamina (requirement fulfilled by the facial artery), and its presence in the media (there are not many elastic fibres in the media of the facial artery). Those authors reported the morphologial reason for the low atherosclerotic affectation of the internal mammary artery.

Regarding physiological intimal thickenings, cushions or pads, and as discussed in the results, in our study we did not observe these, even at the emergence of collateral branches. This is important and means that in the facial artery these regulatory mechanisms are at least rare and that their relationship with the development of pathological thickenings is not direct. We cannot state that they do not exist in this artery, but

they are scarce in comparison with other arteries such as in the genital (Moffat, 1957; Kardon et al., 1983; Whyte et al., 1996), and coronary arteries (Velican and Velican, 1976-77; Pesonen et al., 1990). Despite this, intimal thickenings in the facial artery could be more important in their distal sections, although here we did not study this aspect.

We believe that since this artery transports a large and constant supply of blood without undergoing cyclic changes, as the uterine arteries do, and because of the large number of anastomoses, contrary to what happens with the coronary and mesenteric arteries, the facial artery does not need special regulatory mechanisms such as cushions.We thus believe that the regulation of facial flow is given by these anastomoses and by the smooth muscle cells, arranged both transversaly and as columns of longitudinal fibres, which allow rapid elongations, equilibrated with the elastic tissue.

From the morphometric and immunohistochemical points of view, some results merit comment:

Although the causes of the development of atherosclerosis are not sufficiently clear, there is consensus that the changes in arterial walls (migration, proliferation and dedifferentiation of smooth muscle cells) are fundamental: Whyte et al. (1996), Wissler (1976), Grotendorst et al. (1981), Dilley et al. (1987), Fuster and Badimon (1992), McManus et al. (1995) and Libby (1995). It should be stressed that only arteries with a thick media undergo atherosclerosis while small vessels tend to be resistant to this degeneration.

Embryonic smooth muscle cells are in a undifferentiated stage, but due to factors such as cells, blood pressure, an increase in vascular innervation and others as yet unknown, they change their first nature by proliferation and secretion of extracellular matrix (collagen, elastin and others) and differentiation affords them a contractile phenotype, as happens in adult arteries: differentiated, mature, contractile and not very secretory. This phenomenon of differentiation is characterized by the expression of contractile and cytoskeletal proteins, such as α-actin, myosin and desmin. The most specific of these is α-actin. When, for still unknown reasons, the smooth muscle cell returns to a undifferentiated stage, it shows a decrease in contractile and cytoskeletal proteins, an increase in the secretion of extracellular matrix, and a change in its membrane receptors. The dedifferentiated cells that appear in the first stages of atheromatosis again develop their embryonic noncontractile character of migration, proliferation and secretion.

The more intense reaction to α-actin in the media of arteries without thickenings would in our opinion be due to the migration of muscle cells from the muscle layer to the intima. In arteries with intimal thickenings a number of smooth muscle cells would migrate from the media to the intima and hence the detection of α-actin increases in this latter layer and decreases in the other coat. These findings suggest that one of the mechanisms of the development of intimal thickenings would be migration to the subintimal space, this being more important than the mitosis of subintimal muscle cells (although we do not reject, as Stary states, that this mitosis is present), because if subintimal proliferation were the main origin, the antigenic expression of the media would not change in thickened arteries.

We have detected two degrees of reaction: smooth and strong. Both indicate the presence of smooth muscle cells and although they do not distinguish the power of cell contraction, a strong reaction means a higher concentration of antigens. The strong reaction is almost the same in both groups: this means that the media does not loose its fundamental antigenic expression as a contractile phenotype; the decrease in the smooth reaction in thickened arteries could be due to the beginning of dedifferentiation. Cells that have migrated to the subintimal space are able to secrete a large quantity of extracellular matrix (mainly collagen and proteoglycans) and show a weak contractile power (decreased, although present, α-actin reaction). As time progresses, some myocytes can differentiate and contract again (Porreca et al., 1995 and Lawrence et al., 1995), this meaning that the thickening is old.

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