PATHOLOGY ONCOLOGY RESEARCH Vol 9, No 1, 2003

Article is available online at http://www.webio.hu/por/2003/9/1/0042

ARTICLE

Early Atherosclerosis and Chlamydia Pneumoniae Infection in the Coronary Arteries

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In non-atheromatous segments of coronary arteries a sequence of preatherosclerotic changes was identified which consisted of medial thickening followed by intimal thickening. More recently, Chlamydia pneumoniae seropositivity was associated with enhanced intima-media thickness of arteries. In the present study the intimal and medial thickness of coronary artery of young adults were measured, and were correlated with the presence of Chlamydia pneumoniae antigens. Proximal and distal segments of the left anterior descending coronaries (LAD) obtained at autopsy from young adults (15-34 years) were studied. The thickness and cellular density of the intima and of the media without clear-cut atherosclerotic changes were measured by image analysis. The hypertrophy index was calculated as the ratio of cell density and the thickness of the respective layer. Atherosclerotic lesions occurring elsewhere in the same coronary were noted and graded by severity. The presence of Chlamydia pneumoniae verified by immunohistochemistry was correlated with the severity of lesions and with the hypertrophy index. In the proximal segments, atherosclerosis of LAD was associated with the widening of both the intima and the media of lesion free-sites. In the distal coronary segments the proportion of the intimal thickening had a significant association with atherosclerosis. Compared to non-infected arteries, Chlamydia pneumoniae infection was associated with higher hypertrophy index in the intima as well as in the media. The rate of Chlamydia pneumoniae positivity increased with the severity of lesions. As a conclusion: in the LAD coronary, the intimal thickening is the main preatherosclerotic change. Chlamydia pneumoniae may favour arterial wall hypertrophy and plays a role in lesion progression. (Pathology Oncology Research Vol 9, No 1, 42–46, 2003)

Keywords: chlamydia pneumoniae, early, atherosclerosis, hypertrophy

Introduction

Atherosclerosis has been classified as the disease of the intimal layer of the arteries.[13] However, there are some new aspects for approaching this problem if it is accepted that the intimal and medial layer of the vessels is a complex unit.[14,15] In the aorta and the left descending coronary artery (LAD) the occurrence of atheroma and the intima/media ratio increased progressively from 15 to 34 years of age (WHO-PBDAY study).[7] In young people some arterial structural modifications – such as medial hypertrophy – could be a forerunner of atheroma, as medial hypertrophy was found to be associated significantly with the appearance of atheromatous plaques.[14] The hypertrophic media could be the source of intimal smooth muscle cells.

A key event in the development of atherosclerosis is the accumulation of macrophages in the arterial intima. Its possible connection with a chronic inflammatory process that could thicken the arteries is not well clarified.[11] For a missing link the infection of the vasculature with Chlamydia pneumoniae is challenging, as it is more frequently found in atherosclerotic, than in unchanged arteries supporting its role in the pathogenesis of atherosclerosis.[2,8] More recently, clinical investigations reported that seropositivity for Chlamydia pneumoniae correlates with intima-media thickness.[5,9]

In the present study we surveyed the presence of Chlamydia pneumoniae in segments of LAD in young population. We compared the infected and non-infected coronaries by measuring their hypertrophy index. Arterial

Received: Dec 21, 2002; *accepted:* Febr 10, 2003

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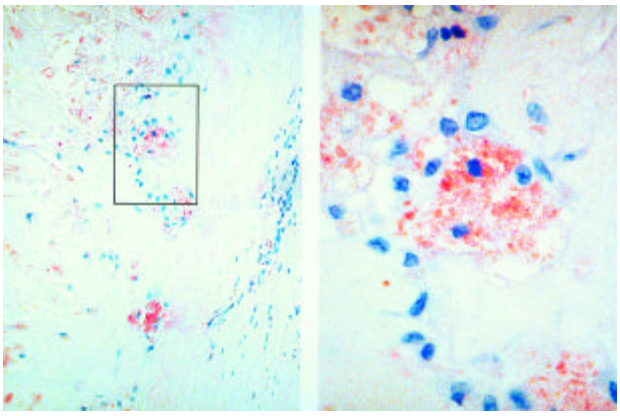

Figure 1. Immunohistochemical detection of Chlamydia pneumoniae antibody RR-402 in coronary artery. Upper: positive monocytes are shown in the thickened intima (100X), in the squdred area foam cells are seen. Bottom: foam cells from the signed area above (400X).

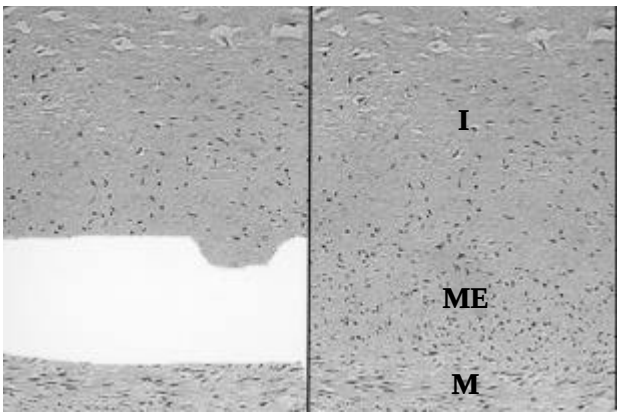

Figure 2. Transversal section of distal LAD coronary. I: upper intima. ME: myoelastic layer. M: media. The thickness of the different layers was computed by dividing their areas and the length of the inner intimal border on the same part of the section. The nuclear density (cellularity) was measured in the upper intima and media as shown in the processed image on the left. 200 X

wall hypertrophy and cell density expressed by a comparable parameter were considered as preatherosclerotic markers.[15] To our knowledge, we are the first to investigate the correlation between immunohistochemically localized Chlamydia pneumoniae infection and the thickness of intima and media.

Material and methods

Left anterior descending coronary artery (LAD) samples were examined. The specimens were obtained from a WHO study of sudden death cases. (See details in article 7). These were collected from 1, 5 and 7 cm away from the origin of the artery at the time of autopsies. Cases with known atherosclerotic risk factors (hypertension, diabetes, smoking and alcohol consumption) were excluded from this study. Sampling, and most of the histology procedures were published elsewhere.[6,7] Intimal lesions were classified in keeping with the suggestion of the American Heart Association.[12]

The following coronary changes were analysed: Type I structural pattern showing different grades of *intimal thickening.* Here we included also *unchanged* arteries. Type II lesions, *the intimal thickening with lipid laden foam cell accumulation* (i.e. fatty streaks). Type III and Type IV are atheromatous lesions. Type I and Type II were included in a morphometric study. (See below). The arterial segments of Type III and Type IV were not considered for further morphometric analysis.

1. Morphometric analysis was performed on a total of 90 samples from 30 cases. These samples were divided further into two subgroups: **A** arteries: samples derived from cases that elsewhere in the coronary had atherosclerotic plaques, and **non-A** arteries: samples from those cases where changes of the coronary system (even far away from the measured site) did not progress to plaque formation. Image analysis was performed on a 4 µm thick H&E stained transversal coronary sections of selected **A** and **non-A** arterial samples. The thickness of the different layers (intima and media) was computed by measuring their areas and the length of the inner intimal surface by image analysis (Scion Image for Windows version Beta 4.0.2., Scion Corporation) at sites of the most flattened lamina elastica interna on the respective part of the section.

2. For the identification of Chlamydia pneumoniae antigens immunohistochemical reactions (TWAR monoclonal antibody RR-402) were performed in consecutive sections on coronary artery samples of 74 cases of the WHO study showing different intimal changes (*Figure 1*). These immunochemical reactions were evaluated semiquantitatively (negative or positive).

3. Morphometric analysis was performed on 8 samples (4 positive and 4 negative for Chlamydia pneumoniae antigens) from 1 cm distally to the origin of the LAD arteries, showing only type I and type II lesions with no atherosclerosis elsewhere (**non-A** arteries) of the above 74 samples. The thickness of the upper intima (intima without the myoelastic layer) and the media was computed as described above. For the measurement of hypertrophy index we worked out a new method in accordance with Tracy.[15] The area of the different layers was measured by image analysis (Scion Image for Windows), and nuclear density (cellularity) was determined for each of the layers. Hypertrophy index was expressed by the ratio of the average thickness and the cell density of that layer. The myoelastic layer, which is an unstable, and probably continuously transforming tissue defined by high cellularity at the

Table 1. **Mean thickness of media and intima and the relative intimal thickness of coronary artery segments with and without atherosclerotic lesions**

Sample site	LAD 1		LAD 5		LAD 7	
Atherosclerosis	*A*	*non A*	*A*	*non A*	*A*	*non A*
n	15	15	15	15	15	15
M (um) ± SEM	149 ± 17	**115** ± 19	109 ± 18	101 ± 11	79 ± 13	92 ± 8
I (um) ± SEM	153 ± 11	**123** ± 11	91 ± 11	70 ± 7	44 ± 7	30 ± 9
I/(I+M) % ± SEM	50 ± 3	51 ± 3	**45** ± 3	**38** ± 4	**36** ± 3	**22** ± 3

A: there is atherosclerosis elsewhere, **non A**: there is no atherosclerosis elsewhere in the coronary. Significant widening of each layer in the proximal segment and relative intimal thickness increase distally as a correlation with atherosclerosis (**A** versus **nonA**). Significant value pairs (P< 0,05) are bold printed. M means media, I means intima, SEM (standard error of means).

medial border of the intima was excluded from the above measurement of hypertrophy index (*Figure 2*). The thickness and the hypertrophy index of the both layer were correlated with the results of Chlamydial immunohistochemical reactions.

ANOVA was used for statistical analysis followed by the pair wise comparison of the group means. Correlations of data were counted by Contingency analysis. The significance level of all tests was set to 0,05.

Results

At the proximal 1 cm segment of LAD both the intima and the media was thickened, when atherosclerosis was present elsewhere along the coronary (**"A**"). The relative intimal thickness is not predictive for atherosclerosis at the proximal segments of LAD. At the segments of 5 and 7 cm distal from the origin of LAD the relative intimal thickness increases in correlation with atherosclerosis elsewhere (*Table 1*).

Twenty five out of the 74 coronary arteries were positive for C. pneumoniae by immunohistochemistry (39%): 8 of 42 arteries with mild disease (19%), and 17 of 32 arteries with advanced lesions (53%) [Type I lesion: 1 out of 19 (5%), type II lesion: 7 out of 23 (30%) type III lesion: 6 out of 15 (40%) and type IV-VI: 11 out of 17 (65%)] (*Table 2*). Advanced lesions were positive for C. pneumoniae significantly more often than early lesions by contingency analysis (p<0,05).

Table 2. **Chlamydial antigen positivity of different preatherosclerotic and atherosclerotic lesions in LAD**

Lesions	*Type I*	*Type II*	*Type III*	*Type IV*
Total N	19	23	15	17
positive	1	7	6	11
% positive	5	30	40	65

The rate of positivity increases significantly (P< 0,05) with the severity of lesions (from type I to IV). P< 0,05 of bold printed percent values.

The presence of Chlamydia pneumoniae antigens was correlated with an upper intimal and medial thickness as well as the hypertrophy index of both the intimal and the medial layer of the proximal segments of LAD (*Table 3*). Upper intimal and medial thickening seems to be uninvolved in the process. Chlamydial infection is associated with higher hypertrophy index (increased intercellular matrix) in the upper intima as well as in the media. There were no similar changes in the myoelastic layer.

Discussion

Intimal thickenings (adaptive thickening) are thought to predispose to atheroma.[12] More recently, the thickening of the medial layer became in the centre of interest. *Tracy at al.* examined longitudinally opened right coronary arteries and found that coronary arteries that contain atheroma tend to possess a media of greater thickness than those that lack atheroma.[14,15] They declare that a fibroplastic thickening of the intima throughout of the right coronary artery was preceded by medial hypertrophy. In our study thickened intima and/or media of the proximal coronaries were associated frequently with atheromatous changes. However, in the distal LAD we found only the intimal thickening as significant candidate for preatherosclerotic lesion that precedes the formation of a plaque.

These changes can be explained by increased synthesis of intercellular matrix and/or smooth muscle cell hypertrophy and/or hyperplasia. In our study, the cell density in the upper intima and in the media did not increase related to atherosclerosis and this would suggest that the increase in thickness of the different vascular layers could be attributed to a balanced cell proliferation and increase in the amount of extracellular matrix.

Table 3. **Thickness and hypertrophy index of the segments of positive and negative Chlamydia pneumoniae immunhistochemistry**

Chlamydia pneumoniae	*negative*	*positive*
Upper intimal thickness (μm)	104 ± 21	119 ± 17
Media thickness (μm)	117 ± 11	129 ± 15
Hypertrophy index of intima (μm^3)	27781 ± 2166	42889± 1034
Hypertrophy index of media (μm^3)	26033 ± 5471	39952 ± 5581

Increased average thickness of the upper intima and media is visible in Chlamydia positive cases. Hypertrophy index of both the intima and the media were significantly increased in Chlamydia positive versus Chlamydia negative coronaries. Significant value pairs (P< 0,05) are bold printed. ± values mean SEM (standard error of mean).

The difference in the intensity of intimal and medial thickening in the proximal and distal LAD may be related to the different structure and function of these segments. Proximal segments are more elastic and changes are resembled to those in the aorta, where medial thickening precedes intimal thickening. The phenomenon of medial growth was observed by us in the aorta and preceded significant intimal thickening and the occurrence of atherosclerotic lesions (nonpublished data).

However, the answer to the question how medial hypertrophy could be linked to atherosclerotic intimal changes is missing. It is not known whether changes of the medial smooth muscle cells influence the atherosclerotic process in the intima, or smooth muscle cells migrate and proliferate in the intima – at sites defined as the myoelastic layer -or the fire-trap of atherosclerosis lays only in the intima and the medial changes being only the consequences of the intimal changes.

Over the last years the role of C. pneumoniae in the development of atherosclerosis has been widely discussed.[1,3-5,8,9] In accordance with the literature, we found a significant correlation between advanced atherosclerotic changes and C. pneumoniae antigen positivity. However, there is no conclusive evidence yet that C. pneumoniae causes atherosclerosis or acute coronary syndromes. Clinical studies reported coronary vessel wall thickening in cases of C. seropositivity.[5,9] Our data presented in this report show that there is a significant correlation between the C. pneumoniae infection and intimal and medial hypertrophy. Chlamydial infection is associated with higher hypertrophy index – mainly increased intercellular matrix – in the upper intima as well as in the media. Thus, our results support that C. pneumoniae may have a role in the lesion progression. C. pneumoniae might disturb the normal regulatory mechanism in the vessels and may initiate a chronic inflammation. Strengthening this premise, C. pneumoniae has been found to upregulate the secretion of gelatinase by human macrophages,[16] and induce the production of monocyte chemotactic protein and interleukin-8.[10] Gelatinase is a metalloproteinase, which degrade collagen fibres. By degrading collagen, C. pneumoniae facilitate the migration of monocytes from the blood to the intima, and probably facilitate the migration of smooth muscle cells from the media to the intima. The production of monocyte chemotactic protein and interleukin-8 also attract monocytes and smooth muscle cells. Regarding our results, we hypothesize that C. pneumoniae may have a stimulatory effect on the smooth muscle cells both of the intima and media to induce extracellular matrix production. By such influences C. pneumoniae may trigger or accelerate atherosclerotic changes.

In conclusion in the proximal coronary both the intimal and medial thickening, and in the distal coronary only the intimal thickening are to be considered as early atherosclerotic changes. C. pneumoniae is more frequently localized in plaques than in unchanged or preatherosclerotic intimas. Its presence is coincident with hypertrophy and increased extracellular matrix of the vascular segments around atherosclerotic lesions, which points to its roles in lesion development and progression.

Acknowledgements

The authors thank Mr. Csaba Lódi for the morphometric measurements and Mrs. Agnes Szik for the excellent technical help. This work was supported by Hungarian National Grants OTKA 1082, and a Ph.D. grant (E.H.).

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