Great Amount of C. pneumoniae in Ruptured Plaque Vessel Segments at autopsy. A Comparative Study with Stable Plaques

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We have previously demonstrated that unstable atheromas are larger than stable ones, and more frequently have positive remodeling. Adventitial inflammation, disappearance of collagen fibrosis and neovascularization are associated with plaque instability and positive remodeling of the vessel. The inflammatory infiltrate in the adventitia is more intense than the inflammation inside the plaque, and this could support the hypothesis that the adventitia may be the main entrance of some infectious agents. We concluded that the unstable plaque is associated with pan-arteritis, which frequently evolve to aneurysmatic vessel enlargement. Such positive vessel remodeling may favour the development of larger fat plaques and plaque instability.

In the present work we looked for C. pneumoniae in the adventitia and in the plaque, using different techniques for detection of C. pneumoniae in situ in unstable and stable plaques in order to clarify whether C. pneumoniae is involved in the etiopathogenesis of such adventitial inflammation in unstable plaques.

Methods

Three groups of necropsy atheromatous coronary lesions were retrospectively studied: Group A - 11 ruptured thrombosed plaques responsible for fatal acute myocardial infarction from 11 patients; Group B - 11 stable plaques from the same patients of group A, presenting similar grade of obstruction but in another coronary branch; Group C - 11 stable plaque from 11 distinct patients who were submitted to elective bypass surgery due to stable angina and did not die due to acute myocardial infarction.

Serial paraffin embedded 5 µm-thick sections were performed in order to detect C. pneumoniae, using 3 techniques- Macchiavello's method (modified); Immunohistochemistry (monoclonal antibody, DAKO Co. USA); In situ hybridization (Oligonucleotide probe end-labeled with biotin, synthesized by GIBCO-BRL, USA). The amount of C. pneumoniae positive (CP+) cells was graded in 0 (absence); 1+ (scarcely CP+ cells), 2+ (moderate number of CP+ cells) and 3+ (foci with many CP+ cells) and 4+ (many foci with a lot of CP+ cells), in slides stained by immunohistochemistry technique and Macchiavello’s...
method. Both methods demonstrated the same amount of CP+ cells.

Two additional recent cases were also studied by electron microscopy and confocal laser microscopy in order to certify that the positivity for C. pneumoniae in macrophages, fibroblasts and smooth muscle cells detected by immunohistochemistry, histochemistry and in situ hybridization were reliable and specific. These two additional cases were from patients who died due to acute myocardial infarction and whose coronary arteries were perfused with formalin. The culprit lesion was detected macroscopically and selected for study through the 5 above described techniques of C. pneumoniae diagnosis. These two cases were not included in the comparative study of the 3 groups previously described.

The quantification of lymphocytes have already been performed in a study with the same cases. These data were used to test the correlation between number of inflammatory cells and score of CP+ cells.

Results

All of the techniques showed many fibroblasts and macrophages positive for C. pneumoniae in adventitia (Figures 1, 2, 3, 4 and 5), in the adventitia of ruptured thrombosed plaque segments. There were also many positive macrophages at the base of the plaque. The frequency of C. pneumoniae detection was very high in groups A, B and C: 100%; 100% and 82% and the mean scores of CP+ cells were 2.73; 1.55; 1.09 respectively. There was a significantly higher amount of CP+ cells (t test) in Group A than Group B (p<0.005) and C (p<0.001), but no significant difference between Groups B and C. There was no linear correlation between the amount of CP+ cells and number of inflammatory cells. However, there was a significant positive association between high score (2 or 3) of CP+ cells and moderate or severe (>15 lymphocytes/mm²) adventitial inflammation (p<0.05- Chi-2 test).

Discussion

Most of clinical and epidemiological trials have pointed to an influence of C. pneumoniae in acute myocardial

Fig. 1 - Electron micrography of an unstable plaque arterial segment showing one adventitial fibroblast containing many intracellular C. pneumoniae elementary bodies, the infective form of the bacteria (A- original magnification X 4200). A closer view is shown in B; original magnification X10000.

Fig. 2 - Electron micrography of adventitial cells presenting forms of C. pneumoniae (CP- arrows); original magnification X 2600.

Fig. 3 - Adventitial inflammatory infiltrate from an unstable plaque arterial segment exhibiting a macrophage labeled for C. pneumoniae (arrow) by immunohistochemical reaction (red granules); original magnification X1000.
infarction or unstable angina. However, the lack of morphological data demonstrating presence of C. pneumoniae in situ in the plaque has led many authors to attribute the inflammation in the plaque to autoimmune processes, questioning the direct role of the bacteria or pointing to an indirect action of it by heat shock protein pathway in the development of plaque instability.

Other authors have recently demonstrated CP membrane protein positivity by immunohistochemistry not only in the atheromatous plaque but also in the adventitia from old patients. In the present work, we reported for the first time such a significantly larger amount of CP-positive cells in ruptured fatal thrombosed plaques than unstable ones. Moreover, we could demonstrate by electron microscopy that the intact bacteria (not only their fragments) are present mainly in the adventitial layer. Moderate to severe inflammation is associated with high numbers of parasitized cells. These results strongly favor the concept that C. pneumoniae is directly involved in the development of adventitial plaque inflammation (pan-arteritis), leading to plaque rupture. A high frequency of C. pneumoniae in all groups suggests its involvement in the pathogenesis of atherosclerosis.

**Conclusion** - Fatal vulnerable atheromatous plaques are associated with larger amount of C. pneumoniae + cells and severe inflammation in the plaque and adventitia, strongly suggesting a direct pathogenic involvement of C. pneumoniae in the rupture of the atheromatous plaque and development of acute myocardial infarction.

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**References**

7. Bezerra HG, Higuchi ML, Palomino S, Silvestre J, Gutierrez PS, Ramires JAF. Atheromas that cause fatal thrombosis are larger and have greater compensatory enlargement than equi-stenotic plaques in the same coronary tree. Circulation 1999; 100: I251.