Properties of calcium-containing microparticles formed in the process of biomineralization of the human aortic wall

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Abstract. The results of the study of human aortic walls, subjected to different stages of mineralization are presented. By means of scanning electron microscopy and X-ray microanalysis the morphology, elemental composition and characteristics of the mineral component localization were investigated. The key differences in the initial, intermediate and final stages of pathological mineralization of the aorta wall were identified. The data obtained may be useful in describing the mechanism of biomineral deposits formation in human body.

1. Introduction
The pathological mineralization in human body can usually be the sight of such diseases as: kidney stone disease, arthritis, arthrosis, atherosclerosis, calcium thesaurismosis. Mineralization phenomenon is the deposition of minerals in the organs and tissues. The development of this process is facilitated by the alteration in the ratio between specific types of proteins, the activity of hydrogen ions in biological fluids, disorders of calcium metabolism, enzymatic and other factors.

It is commonly considered that first stages of atherosclerosis are associated with the deterioration of the vascular wall [1-7]. For example, immune complexes may cause such deterioration. The immune complexes are formed at the time when there is an excess of body antigens (infections, viruses, bacteria) and cause local inflammation of soft tissues in the body [1-2]. Hypercholesterolemia leads to disruption of condition of the vascular wall also. It is accompanied by increased levels low-density lipoprotein (LDL) in the blood. Oxidized LDL exerts toxic effect on endothelial cells. In response, the monocyte clusters are trying to eliminate inflammation, but in complex with LDL, are transformed into foam cells, subsequently they accumulate in the vascular wall [1,3-4]. The toxic factors such as smoking, radiation, foreign chemicals may result in oxidative stress for endothelial cells. Oxidant molecules have one or more unpaired electrons, and aspire compensate their quantity by means of endothelial cells. As a result, the endothelial cells become unstable and endothelial layer of the aortic wall structure is damaged. Toxic factors cause disorder in the metabolism of cells. It may be accompanied by a mutation, apoptosis or cell proliferation [2,5]. Hemodynamic loads lead to wear, disruption properties and elasticity of the vascular wall tissue. The sudden change in blood pressure causes the vascular spasm. This results in nutrition disturbance of the large arteries as well as tissues of vital organs [6]. Vascular spasm occurs also with stress, causing disruption of the receptor system and subsequent failure in the transmission of nerve impulses and the protective functions of the organism [7].
The only violation of the state of the vascular wall, of course, is unlikely to lead to development of its atherosclerotic lesions. Atherosclerosis can spread like an avalanche, as a result of chronic inflammation of soft tissues and organs, as well as influenced of promotor factor. The most important one of such factors is adverse genetic code. Thus, for example, may be deteriorated production of some enzymes, which should participate in the removing died off cells. As a result these cells accumulation in the body and their subsequent mineralization takes place. Metabolic disorders and acid-base balance, increased concentrations mineral ions in the blood and, as a result, elution of ions from the bone may occur if we have unbalanced diet and lack of physical activity. Under conditions of high acidity the one type of mineral deposits is formed, while in alkaline environment are formed rather different deposits [1,6-7].

Final stages of the disease are related with deposition of calcium salts in the vascular wall, resulting in aorta elasticity loss in fully mineralized tissues. The areas of calcification may constitute lesions, clearly visible to the naked eye. However, these lesions are generally composed of minute particles which are detectable by means of electron microscopy only.

The main role in mineralization process in a human body and particularly in soft tissues belongs to calcium containing nanoparticles [8-10]. Sizes of the particles varies in 50-400 nm range. But till now the mechanism of their nucleation, their properties and characteristics remain completely unclear.

To clarify the role of these particles in the development of cardiovascular disease the careful analysis of their morphological features, the elemental and phase composition as well as the characteristics of their localization at different stages of formation of mineral deposits in the soft tissues of the human cardiovascular system is required.

This work presents the results on study of morphology, location and composition of the mineral deposits in the specimens of human aortic walls, obtained by the methods of electron microscopy and X-ray microanalysis at various stages of their damage by mineral compounds.

2. Materials and methods

As a samples of tissue of the human cardiovascular system were used aorta sections, exposed to various degrees of damage by mineral compounds and with varying degree of the aortic wall integrity. Tissue samples have been stored in formalin and transported within one day. Further the samples were washed by an isotonic solution pH = 7.4 and were stored in the same solution within a few days in the refrigerator at a temperature between 1-5.

For samples study by means of electron microscopy and X-ray microanalysis they were frozen in liquid nitrogen for about an hour, followed by preparation of several cleavages of the samples. Obtained samples were fixed by a glue on a silicon substrate cleaved surface up. Finaly the samples were washed in 0.1 M citrate buffer pH = 2 for 15 minutes.

All of the samples were examined at electron-ion microscope Quanta 3D 200i in a low-vacuum mode. Morphological characteristics of mineralized sections of the samples, a feature of the location and composition of the mineral components in the aortic wall were thoroughly studied. To produce the image both the secondary electron detector and the detector of the backscattered electrons were used.

3. Results and Discussion

The results obtained evidence that most probably the biomineral formation of deposits in the soft tissues of the human cardiovascular system is a multistage process. The initial stage of biomineralization features the presence of solitary mineral spherical particles of 50 nm to 2 mm in size in the media layer of the vessel wall (Figure 1).
**Figure 1.** SEM microphotographs of human aortic walls at initial stages of pathological mineralization:

a) - transverse cleavage of aortic wall with the initial symptoms of pathological mineralization. Magnification - 600. (BSED - back-scattered electrons detector allows to reveal the area of mineralization at low magnifications);
b) - micro and nanoparticles in the media layers of the aortic wall (this is the first sign of pathological mineralization) BSED;
c-d) - the same domain of the media layer of aortic wall. Magnification - 650 (c - LFD - Live fiber detector, d) - BSED);
e-f) - the same domain (the rectangular area in Figure 1d) of the media layer of aortic wall obtained at magnification - 4000 (e - LFD, f - BSED)

The next stage is associated with appearing of particle clusters localized in aorta wall and increase of particles volume. The typical size of the mineral particles at this stage reaches several micrometers. The clusters have a size varying from 5 micrometers to hundreds of micrometers (Figure 3). Outbreaks of extensive mineralization are predominantly arranged along the fibers (Figure 3b), between them (Figure 2, 3g-h) and inside them (Figure 3c-f). Mineralization at intermediate stages takes the form of thin layers, located between the fibers of the media layer. (Figure 2a).
Figure 2. SEM microphotograph and X-ray map of elements distribution in human aortic walls at intermediate stages of the mineralization:

a) - transverse cleavage of aortic wall with the sign of pathological mineralization. Magnification - 100.

SE - electron image of a media domain of aortic wall and map of calcium (Ca), phosphorus (P), sulfur (S), carbon (C) and oxygen (O). The bright domain on the map is a localization place of the corresponding element in the sample.

Figure 3. SEM microphotographs of human aortic walls at intermediate stages of the mineralization.

(a-b) The same domain of the media layer of aortic wall with magnification 1000 (a-LFD, b-BSED); (c-d) The rectangular area highlighted in Figure 3a with magnification 4000 (c - LFD, d - BSED); (e-f) The same domain of the media layer of aortic wall at magnification 3250 (e- LFD, f- BSED); (g) The domain of the media layer of aortic wall at magnification 3250 (BSED); (h) The rectangular area highlighted in Figure 3g at magnification 12500.

The final stages are characterized by increasing volume of mineralized areas and the dense packs of mineral particles, measuring several micrometers. Subsequently, the mineralized areas are identified as whole mineral agglomerates (Figure 4b). It can also be noted, that the initial stages of mineralization...
are observed in the media layer, but at a later stage the shift of the extensive mineralization towards the aortic intima is observed (Figure 4c).

Figure 4. SEM microphotographs of human aortic walls at final stages of pathological mineralization:

- a-b) the same domain of the media layer of aortic wall at magnification 100 (intima is at the top of the image, below is a layer of the media, and at the bottom of image is adventiscya aortic wall)
- b) BSED (more light domains are the places of mineralization localization).
- c) – a cleavage of the aortic wall at the site of mineral aggregate at magnification 500. The arrow indicates the direction from the intima to the media (LFD).
- d) the conventional boundary of transition from the intima to the media at the late stage of mineralization (LFD).

It remains unclear why the mineralization is initiated and proceeds along the muscle fibers of the media, and is often observed in the form of layers disposed between them? Media is composed of aortic smooth muscle cells, and extracellular matrix proteins, first of all of collagen and elastin. Mineral micro- and nanoparticles, ranging in size from 50 nm to 2 microns were detected on the surface of the protein fibers (Figure 1b, 1f); between them (Figure 2, 3g-h); and also in the volume of fibers (Figure 3c-f).

The hotbeds of the initial stages of mineralization of aortic wall lie in a media layer. Media of aortic wall has a high density distribution of smooth-muscle cells as compared with the intima [11]. In turn, in the processes of cellular metabolism the regulation of calcium level is the responsibility of Ca-binding proteins such as calmodulin, troponin C and parvalbumin [12]. Violation of secretion of Ca-binding proteins or of its structure inevitably results in violation of cell homeostasis. The cell consists of various organelles (nucleus, nucleolus, vesicles, and lysosomes) which have a spherical shape. As a result of calcium excess in the cell, these organelles may serve as the matrix for the deposition of mineral components. The proteins, which make up the cells, in this process are most probably degrading. During the protein molecules destruction, the sulfide bonds, that support the protein structure, are torn in the first turn. Apparently, at destruction of sulfide bonds sulfur somehow is deduced from affected area when the sulfide bonds are broken, because it is not detected at the later stages of mineralization.

4. Conclusion

To summarize let us mention that the key differences in the initial, intermediate and final stages of pathological mineralization of the aorta wall were revealed. Combining the obtained results, it can be argued that the aortic mineralization indicated in the layer media. The size of the mineral particles detected in the local conglomeration is different. Dimensions of local conglomerations correlate well with the size of the smooth muscle cells. Dimensions of single mineral particles correlate well with the size of the cell organelles. In this regard, one can assume that the mineralization begins with the pathology of the homeostasis of a smooth muscle cells. The presence of extensive areas of
mineralization can be explained, for example, by the disturbances in several adjacent cells. The data obtained may be useful in revealing the mechanism of biomineral deposits formation in human body.

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