Biomineralogy of Osteoporosis

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ABSTRACT

Biomineralogical examination of fine blood vessels from femur heads affected by osteoporosis (removed during total alloplastic surgery of hip joint) was performed, using SEM-EDS method. The examination was focused on biomineralization of the internal walls of micro-arteries. Results document the presence of biomineralization in the internal walls of micro arteries in the areas affected by osteoporosis. Observed phenomenon confirms that blood vessels in the areas of femur heads affected by osteoporosis do not function properly. Such phenomenon leads to changes in local chemical environment of bone caused by reducing the transport of oxygen and other components necessary for normal functioning of bone cells. Such reduction of oxygen is the cause of changes in local pH. Since bone hydroxyapatite is not stable at pH<6.6, it begins to be dissolved in bone bars (collagen-apatite structure) and removed from parts of the structure of bone. Removal of hydroxyapatite reduces bone mass and introduces free Ca²⁺ and PO₄³⁻ from bone to bloodstream. Described phenomenon accelerates the process of bone blood vessels blockage, in effect accelerating osteoporosis. Because of limited funds and difficulty in obtaining material for examination, the analysis is not statistical.

Key words: Micro arteries, biomineralization, osteoporosis.

INTRODUCTION

Analyzing the details of the osteoporosis process is essential not only because of its destructive impact on bone structure. In addition to the destruction of bones, osteoporosis is a mechanism that causes elements to be released from the affected bone structure and introduced into soft tissue (Pawlikowski, 2014). Ions released from the bone are transported through the circulatory system, and their introduction into soft tissue occurs in spots called crystallization centers. Depending on where those centers appear, that is where calcification develops. Apart from calcification, other substances may concentrate in those spots as well - cholesterol, lipids and fats etc (Klita and Pawlikowski, 1983; Pawlikowski, 1987, 1991a, b, c, d; Niedźwiedzki et al., 1987a, b; Niedźwiedzki et al., 1993; Pawlikowski and Pfitzner, 1992; Pawlikowski, 1993, 1994, 1995, 1998, 1999, 2003, 2004, 2011a, b, 2013, 2014; Bieniek et al., 2011; Pawlikowski et al., 2015). Development of new examination methods, including methods using an electron beam has allowed for examination of micro blood vessels. This work presents the examination results of such vessels localized in bones and their immediate vicinity. The research is not statistical in nature because of limited funds and difficulty in obtaining test material.

MATERIALS AND METHODS

Research material consists of fragments of micro-arteries obtained from four femur heads removed during total hip alloplasty. Materials (from men aged 30, 50, 56 and 64) were obtained. The femur heads were cut into 1 cm slides in accordance with the drawing presented in Figure 1. Femur head slices underwent densitometry analysis with
the use of Hologic Horizon W, software version APEX and Delphi W. Hologic, software version 11:1:7. Samples for further testing were taken from the spots in the bone slices where the densitometry indicated significant thinning of the bone structure. Blood vessels were prepared under a Moticm. 07-100477 microscope of Chinese production in 400x magnification.

Blood vessel fragments removed from the bone slices were preserved in buffered formalin. Before each test, they were repeatedly rinsed with distilled water and ethanol, and before SEM testing, dried in vacuum. Scanning microscope Geol 5400 of Japanese production and electron scanning microscope (SEM) by FEI Quanta 200FEG were used. Chemical analysis of mineralization within a micro-area of fine blood vessels' internal walls was performed using electron beam detector EDS that is part of the microscope. The analysis was performed in a low vacuum setting.

RESULTS

The entire tested femur heads revealed significant cartilage defects and damage. Example is shown in Figure 2. Examination of cartilage from each femur head performed using mineralogical methods showed significant amounts of calcium and phosphorus, as well as other elements in remaining fragments proving its serious mineralization (Bieniek et al., 2011; Pawlikowski et al., 2015).

Densitometry of the slices obtained from the examined femur head indicated the multisport character of the changes connected with osteoporosis, which is visible as partial necrosis within bone. Figure 3 shows an example of densitometric photographs of respective slices of femur head from a 30-year-old man.

Microscopic examination of places showing bone bars loss was performed in all slices of all heads. Small fragments of micro-vessels were taken from those places for the next examination. In turn, femur head slices were deproteinized using diluted solutions of H2O2 and KOH. Deproteinization was done in the mixture of those solutions in the temperature of 60°C until all the substances were removed from the spaces between the bone bars. Obtained material was examined under a microscope to properly analyze the degree of changes in bone structure where densitometry had shown a significant bone loss.

Microscopic observations confirmed the results of densitometry. Within the dark areas from the densitometric photographs of the femur head slices, the bone structure turned out to be thinned. Thinning of the bone bars structure is not homogenous: in one bone slice, the structure may be dense and well-preserved in one spot and thinned in others. The thinned areas are places where the bone bars are less numerous or even, in some micro-areas, gone entirely (Figure 4).

More detailed analysis of the deproteinized bones was performed with a scanning microscope. That analysis showed that in the area where the bone bars structure was thinned, the bone bars themselves are partially destroyed. There are tiny fissures and "washed out" areas that indicate weakening of their structure. It is apparent both around the haversian canals and in the bone bars structure itself.

Figure 1. Cutting of femur heads prepared for examination (By A. Bieniek).
Figure 2. Surface of examined femur head. Joint cartilage is damaged down to the bone. The arrow shows the point of contact between cartilage and bone.

Figure 3. Sequence of densitometric photographs of examined femur head slices from a 30-year-old man. Dark spots are places of bone loss where micro-vessels were collected for next examinations (By A. Bieniek).
Figure 4. Different phases of bone preservation - destruction observed within one slice of one femur head (56-year-old man). Scale – mm.

Figure 5. A - microscopic (SEM) photograph of a fragment of deproteinized bone. Visible is haversian canal and its immediate vicinity; B: microscopic (SEM) photograph of a fragment of bone bar in the deproteinized bone. Visible are defects of the bone bar structure - fissures and breaks.

Examination of internal wall fragments from blood vessels showed presence of aggregative forms not larger than a few millimeters (Figure 6). Each "crystal" is typically smaller than a micrometer. The aggregations are built from non-mineral phases (fats and cholesterol) as well as inorganic compounds, which, analyzed by the EDS method proved to contain phosphorus and calcium (Figure 3).
Figure 6. A: Organic aggregations (fats or cholesterol) on a wall of a micro-artery from a femur head. SEM; B: EDS spectrum of the phosphorous aggregation from the wall of a micro-artery taken from a femur head SEM-EDS.

Microscopic examination performed with a scanning microscope, as well as EDS examination indicate that typically, aggregations of both organic substances and phosphates are found on the micro-artery walls and coexist in various proportions.

DISCUSSION

Next to unchanged areas of spongy bone (Figure 7A) in the examined femur heads, areas of thinned structure appeared. Densitometry indicates irregular appearance of necrotized spots developing towards osteoporosis. Examined micro-blood vessels taken from the bone revealed presence of fats and phosphates both on the internal walls and, less frequently, in the wall itself (Figure 7B). Their presence in the micro-arteries reduces the inner diameter of the blood vessels, hindering blood flow and, consequently, the process of supplying oxygen and polysaccharides to bone cells.

Such situation causes changes in the proportions of oxygen to CO\(_2\) in bone environment. Disturbance in the proportion of supplied oxygen to CO\(_2\) produced by the bone cells in their life processes reacts with the local water, creating easily dissociating carbonic acid (H\(_2\)CO\(_3\)) that lowers the local pH. Drop in pH below the solubility product of hydroxyapatite appearing among collagen fibers (in bone bars) causes slow dissolution. In addition, collagen is also altered. Products of that alteration additionally acidify the environment, accelerating the process of bone bars’ destruction (Figure 7C). That process is initially noticeable (in greater magnification) as losses of bone bars in spongy bone, while in more advances phases it is visible in densitometric photographs as dark spots with clearly diminished absorption of radiation (Figure 7D).

As indicated by the test results presented in this work, one of the main causes of osteoporosis is a dysfunction of tiny arteries in bones. That dysfunction is a result of their partial blockage by substances accumulating both in the micro-artery walls and on their internal walls.

It is still unclear why some blood vessels are blocked and others are not, or what phenomena govern the crystallization of different substances in bone micro-arteries. Undoubtedly, one of the reasons is the presence of crystallization centers in those vessels, where calcification can occur and cholesterol accumulates etc. (Pawlikowski, 2014).

In-depth explanation of those phenomena requires further studies, especially due to a potential chance of preventing the mineralization of micro-arteries (preventive treatment), as well as, hope for finding ways of dissolving the existing accretions (Pawlikowski, 1987, 1999; Pawlikowski and Ryskala, 1991; Pawlikowski and Pfitzner, 1992, 1995a, b, 1999; Lipinicka et al., 2003; Niedźwiedzki et al., 1995; Pawlikowski et al., 1994, 1995, 1996, 1999; Pawlikowski and Niedźwiedzki, 2002; Pfitzner et al., 2003). Even a little progress in that direction is impossible to overestimate from the perspective of human life and health.

Conclusions

The following conclusions drawn from the research are:

1. Obtained data suggest that osteoporosis is the result of bad functioning of blocked micro-arteries present in bones.
2. Bone micro-vessels affected by bio-mineralization are easily blocked, similarly to peripheral vessels present, for example, in skin.
3. Blockage of micro-vessels caused by a build-up of organic
Figure 7. A: Upper: Schematic drawing of a normal structure of spongy bone; lower: process of oxygen and nutrient transport to bone cells in a normal spongy bone. B: Upper: Location of mineralization on and in the internal walls of the bone micro-artery. C: Outline of phenomena within the spots in bone bars where increase in carbon dioxide (and carbonic acid) and drop in pH occurred. Elements released during dissolution of bone hydroxyapatite crystals and destruction of collagen fibers are transported out of bone and into circulatory system. It causes thinning of bone bars, followed by their loss. D: Upper: outline drawing of spongy bone structure affected by osteoporosis. Lower: densitometric picture of femur head slices affected in places by necrosis and osteoporosis.

Pawlikowski (2014) states that the reduction of oxygen supply to bone cells causes relative increase of CO₂ (and in consequence H₂CO₃), which leads to lowering of the pH of local environment in the bone structure.

5. pH lowering below 6.6 (Pawlikowski and Niedźwiedzki, 2002; Pawlikowski, 2014) leads to dissolution of bone hydroxyapatite, which is not stable in acidic conditions.

6. Additionally, acidic conditions cause alteration of bone collagen fibers.

7. Alteration of collagen results in generating more H₂CO₃ and, in consequence, acceleration of the process of bone structure destruction.

8. The aforementioned processes are the main reason of spongy bone destruction, that is, reduction of physical parameters of bone.

9. It is essential to remember that osteoporosis is not only a process of bone destruction. The phenomenon includes transfer of elements removed from bones to various soft tissues, resulting in many different diseases and in consequence, death of organism (Pawlikowski, 2014).

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