Nuclear Instruments and Methods in Physics Research B xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Nuclear Instruments and Methods in Physics Research B

journal homepage: www.elsevier.com/locate/nimb

Determination of oxidation state of iron in normal and pathologically altered human aortic valves

J. Czapla-Masztafiak [a,*], G.J. Lis [b], M. Gajda [b], E. Jasek [b], U. Czubek [c], F. Bolechała [d], C. Borca [e], W.M. Kwiatek [a]

[a] Institute of Nuclear Physics PAN, Radzikowskiego 152, 31-342 Kraków, Poland
[b] Department of Histology, Jagiellonian University Medical College, Kopernika 7, 31-034 Kraków, Poland
[c] Department of Coronary Disease, Jagiellonian University Medical College, John Paul II Hospital, Prądnicka 80, 31-202 Kraków, Poland
[d] Department of Forensic Medicine, Jagiellonian University Medical College, Grzegórzecka 16, 31-531 Kraków, Poland
[e] Swiss Light Source, Paul Scherrer Institute, 5232 Villigen PSI, Switzerland

ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form 13 April 2015
Accepted 13 April 2015
Available online xxxx

Keywords:
X-ray absorption spectroscopy
Iron
Aortic valve stenosis

ABSTRACT

In order to investigate changes in chemical state of iron in normal and pathologically altered human aortic valves X-ray absorption spectroscopy was applied. Since Fe is suspected to play detrimental role in aortic valve stenosis pathogenesis the oxidation state of this element has been determined. The experimental material consisted of 10 μm sections of valves excised during routine surgery and from autopsies. The experiment was performed at the MicroXAS beamline of the SLS synchrotron facility in Villigen (Switzerland). The Fe K-edge XANES spectra obtained from tissue samples were carefully analyzed and compared with the spectra of reference compounds containing iron in various chemical structures. The analysis of absorption edge position and shape of the spectra revealed that both chemical forms of iron are presented in valve tissue but Fe^{3+} is the predominant form. Small shift of the absorption edge toward higher energy in the spectra from stenotic valve samples indicates higher content of the Fe^{3+} form in pathological tissue. Such a phenomenon suggests the role of Fenton reaction and reactive oxygen species in the etiology of aortic valve stenosis. The comparison of pre-edge regions of XANES spectra for control and stenotic valve tissue confirmed no differences in local symmetry or spin state of iron in analyzed samples.

1. Introduction

According to the report prepared by European Society of Cardiology in 2003 [1] aortic valve stenosis (AS) is the most common valvular heart disease. AS is characterized by pathological processes, leading to varying degrees of morphological changes of the aortic valve leaflets, including fibrosis, accumulation of lipids, calcification and occasionally bone tissue formation. All these processes result in valve leaflet thickening, calcification and inflexibility and clinical symptoms of aortic valve stenosis which prevalence increase with age [2]. Over the years pathophysiologically the process was described as purely passive degeneration progressively degrading the fine trilaminar valve leaflet architecture. Subsequently its substantial modification abilities have been questioned. However, recent years brought emerging epidemiological, histopathological, and experimental evidences indicating that the process in fact is actively regulated by various cells (e.g. endothelial, inflammatory and activated interstitial cells), cytokines produced by these cells, matrix glycoproteins (e.g. matrix metalloproteinases, bone morphogenetic proteins, tenascin) as well as blood borne mediators (e.g. renin angiotensin system, osteoprotegerin and receptor activator of nuclear factor κB ligand) [3–7]. It is stressed that the histological appearance of stenotic aortic valves and risk factors of the disease resembles atherosclerotic lesions [8].

Since valve replacement is eventually the only treatment applicable for aortic valve disease [9], and grafted bioprosthetic valves seem to undergo similar degeneration as observed in native ones [5], it is extremely important to understand the mechanisms involved in the process and to find out medical therapies able to prevent or slow down its spread.

One of the suggested pathogenic mechanism of AS and other major cardiovascular diseases is the activity of superoxide and other reactive oxygen species (ROS) [8]. It was shown that superoxide concentration is increased in human stenotic aortic valves [10]. Excessive production of ROS can cause damages of DNA,

* Corresponding author.

http://dx.doi.org/10.1016/j.nimb.2015.04.026
0168-583X/

proteins and, which is particularly important in the initiation and progression of AS, in lipid peroxidation. One of the chemical mechanism that leads to ROS production is Fenton's reaction, where the iron ions are involved. Iron is crucial to maintain cellular function and integrity, however, careful homoeostasis is also critical as both iron-deficient and overload states can lead to pathological changes in human body [11]. The role of iron has, inter alia, been confirmed in the process of atherosclerosis [11,12].

In order to optimize Fenton reaction low pH values are required. The rationale for the presence of acidic environment in the aortic valve degeneration comes from observation that the process is significantly associated with inflammation. Inflammatory conditions in various tissues are known to create locally acidic environment. In calcific valve degeneration as well as atherosclerosis high infiltration of macrophages is observed. Activity of these cells, among others, can acidify microenvironment creating optimal milieu for Fenton reaction [13]. Recently we found the population of osteoclastic-like cells in stenotic valves [14]. These monocyte-derived cells typically associated with bone tissue are able to release H+ ions resulting in local acidification and decalcification. What is more, local intravalvular hypoxia as a consequence of pathological valve thickening may results in increased anaerobic metabolism and lactate production and in this way lowering pH in affected tissues.

There are also evidences for the presence of redox-active iron intracellularly in macrophageal lysosomes which are vesicles containing a set of acid hydrolases in naturally low pH (4–5) [15]. Since it was documented in atherosclerotic plaques it is reasonable to suggest that in degenerating valves (a pathology akin to atherosclerosis) similar mechanisms are active as well.

The main objective of the presented study is to compare the oxidation state and chemical environment of iron in stenotic and normal human aortic valves using the possibilities offered by X-ray absorption near edge spectroscopy (XANES). Similar approach has been already used in case of, for example, studies of oxidation state of iron in non-cancerous and cancerous prostate tissue sections [16] and the iron speciation in human nails [17]. Presented analysis is complementary to the distribution studies of selected elements by microscopy and SR-μXRF performed on the same samples [18].

2. Materials and methods

The examined material comprised 4 human aortic valves. Valves were excised during routine surgery and from autopsies. The samples included 2 calcified stenotic valves and 2 normal (non-stenotic) valves. The study protocol was approved by the Bioethical Committee of the Jagiellonian University Medical College and the patients informed consent was obtained.

Tissue serial sections (10 μm-thick) were cut frozen on cryostat, mounted on 3 μm-thick Mylar foil, dried and subjected to measurements. Additional sections were processed to histological hematoxylin and eosin (HE) stained specimens.

Additionally, a set of powder reference compounds (both inorganic and organic), that represented different iron oxidation and different bonding environment, was examined. Powders were thoroughly grinded in a mortar with boron nitride (BN) at a mass ratio of 1:10 in case of inorganic compounds and 1:4 in case of organic one. The proportion of BN and reference compounds was chosen in order to obtain good quality fluorescence spectra. Homogeneous powders were placed into holes of the special plastic holder and placed at the experimental station.

XANES measurements of Fe K-edge were performed at the MicroXAS beamline of the SLS synchrotron facility (PSI, Switzerland), in fluorescence mode, in air. A single element Si(Li) fluorescence detector (Ketek) was used. The energy was tuned with the double crystal monochromator (Si(1 1 1) crystal) and the beam was focused with Kirkpatrick–Baez mirror system. Full Fe K-edge XANES spectra were measured on 5 different points on each of the tissue samples in energy range 7015–7500 eV with acquisition time of 1 s/energy point. In case of stenotic valves points were chosen so as to be near the focal calcifications, what was verified on adjacent routinely stained (HE) histological specimens. The beam was focused to the size of 10 μm × 10 μm and 350 μm × 350 μm in case of tissue samples and reference samples, respectively. In order to obtain good quality data 5 XANES spectra were recorded on each reference sample and merged. In case of tissue samples the number of spectra were limited to 3 at each point to avoid possible radiation damage. The photon flux during measurements was about 2×10^{11} photons/s/μm^2. The collected spectra were carefully examined and no changes in shape nor position were observed between spectra taken at one point.

All the XANES spectra were analyzed using ATHENA software package [19]. The background subtraction from the raw XANES data was performed in ATHENA that determined it by optimizing the low frequency components of Fourier transform of the data. Then the data were normalized to the post-edge part. For reference compounds the self-absorption correction was performed. Despite the fact that the reference compounds have been mixed with boron nitride in order to reduce the concentration of Fe atoms in samples, the obtained XANES spectra shown the influence of self-absorption. For this reason the self-absorption correction algorithm, offered as one of the features in Athena, was applied. The examples of such corrections are presented in Fig. 1.

3. Results and discussion

The first step in the analysis of obtained data was the comparison of Fe K-edge spectra for valve samples and reference samples. The spectra of reference samples with known chemical composition provide information on the shape of XANES spectra and their position on the energy scale, that depends on the chemical environment of iron. In the case of iron the energy position of the absorption edge of the XANES spectrum can be determined by both the oxidation and spin state of the atom [20,21]. The Fe K-edge XANES spectra of reference compounds are presented in Fig. 2.

It can be easily noticed that the shape of individual spectra varies depending on the local chemical environment of the iron in the analyzed compounds. Moreover the energy shift of absorption edge between individual spectra due to various oxidation and spin state of iron (2+ or 3+ oxidation state, low or high spin) is observed.

The comparison of the Fe K-edge XANES spectra obtained for control samples (K1 and K2), stenotic valve samples (S1 and S2) and three of the reference compounds is presented in Fig. 3.

The XANES spectra obtained for the tissue samples are located between the XANES spectra of Fe^{2+} and Fe^{3+} reference compounds but closer to the spectrum of $K_2Zn[Fe(CN)_6]$ that contains iron on 3+ oxidation state. This indicates that both chemical forms of iron are presented in valve tissue but Fe^{3+} is the predominant form, which is in agreement with the literature [22]. The shape of spectra from tissue samples is similar to the one obtained for hemin – the haeme derivative, in which iron has similar chemical environment to the one in haemoglobin, myoglobin or cytochromes, that play very important role in biological systems. Hemin contains haeme structure with trivalent iron coordinated to four nitrogen atoms and chloride ligand, positioned outside the haeme plane. Next shell is built of carbon atoms [23]. In contrary to hemin structure, iron in $K_2Zn[Fe(CN)_6]$ and $Na_2[Fe(CN)_5NO]$ is coordinated directly to carbon atoms of cyanide groups and one nitrogen atom in case of

Please cite this article in press as: J. Czapla-Masztafiak et al., Determination of oxidation state of iron in normal and pathologically altered human aortic valves, Nucl. Instr. Meth. B (2015), http://dx.doi.org/10.1016/j.nimb.2015.04.026