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Spin traps in the detection of free radicals in the blood of patients with ischemia

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Electron Paramagnetic Resonance and a nitrosobenzene spin trap were used to investigate free radicals in the human blood after angioplasty treatment. The nitrosobenzene anion radical was determined using EPR measurements and quantum-mechanical calculations. Differences were observed in the concentration of free radicals before and after angioplasty treatment. These results were compared with myocardium damage parameters (CPK, MB and TnT).

Key words: Electron Paramagnetic Resonance, nitrosobenzene spin trap, angioplasty treatment, free radicals.

Introduction

Recent years has seen an explosion of interest in the production and reactions of free radicals in biological systems and also in the human body. A major role is played here by oxygen free radicals (OFRs). They are being constantly formed in the human body in normal metabolic processes, as a result of the reduction of oxygen to water by the mitochondrial electron transport chain. This reaction is greatly accelerated by superoxide dismutase (SOD), a widely distributed enzyme [1, 2, 3].

Cardiovascular diseases refer to a heterogeneous group of disorders that affect the heart and blood vessels. These diseases are characterized by angina pectoris,

hypertension, congestive heart failure, acute myocardial infarction (heart attacks), stroke, and arrhythmia [8]. Considerable biochemical, physiological and pharmacological data support a connection between free radical reactions, inflammatory states and cardiovascular tissue injury [4, 7, 8].

The aim of this study was to investigate free radicals using a nitrosobenzene spin trap and Electron Paramagnetic Resonance (EPR). The study involved identification of the type of free radicals and determination of their concentration in the conglomerate of spin trap and peripheral blood of patients subjected to angioplasty treatment. The changes in free radical concentration were compared with myocardium damage markers (CPK, MB and TnT).

Material

Sixty people were hospitalized because of acute coronal syndrome and were treated by using primary coronal angioplasty in the Haemodynamics Laboratory at the Swietokrzyskie Cardiology Centre of the Provincial Joint Hospital in Kielce, Poland.

The blood from the peripheral vein was taken directly before and 2 hours after angioplasty treatment to determine biochemical and inflammatory parameters. A resting cardiogram was obtained. Twenty four hours after angioplasty a new blood sample was taken and an ECG examination was performed again.

Methods

The measurements were made with a Bruker EPR EMX-10 X-band (9.4 GHz) spectrometer with a magnetic field second modulation frequency of 100 kHz. Low temperatures were maintained by a Bruker temperature controller ER 4131VT. EPR spectra were recorded at 170 K in a sweep width of 20 mT.

From the blood taken from patients, a 0.2 ml sample was mixed with 4 ml of nitrosobenzene solution immediately after blood collection and divided into three identical samples. After 1 minute these samples were placed in a dewar with liquid nitrogen (77 K) and stored. To check the repeat procedure and to estimate the errors in free radical concentration, every time three samples of blood with spin trap of the same

patient were investigated. The error in free radical concentration in these three control samples for each patient was estimated at about 10%.

The nitrosobenzene spin trap C_6H_5NO, obtained from Aldrich Chemical Co., was used in the measurements. In this experiment, 100 mg of nitrosobenzene were dissolved in 3 ml of methanol CH_3OH and 97 ml of distilled water was added.

The EPR spectra were simulated in a WINEPR SimFonia program from Bruker.

The quantum-mechanical calculations were done in the Gaussian98 program. Geometric structures were optimized with the B3LYP/6-31G**, and the A-tensor was calculated with the B3LYP/EPR-II method also implemented in the Gaussian98 program.

Results

At the beginning, the EPR spectra of the blood with a spin trap were recorded. All EPR spectra of such a conglomerate were similar in shape, but they differed in their EPR signal intensities. Exemplary spectra of a blood-spin trap conglomerate are shown in Figure 1: before, 2 hours and 24 hours after angioplasty. The biggest signal and the highest radical concentration were observed 2 hours after angioplasty (Figure 1), and then it decreased.

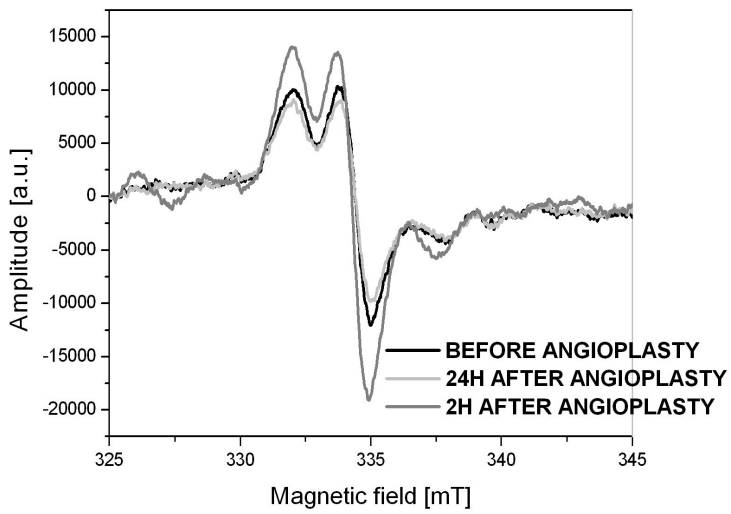

Figure 1. The EPR spectra of blood-spin trap conglomerate recorded at 170K

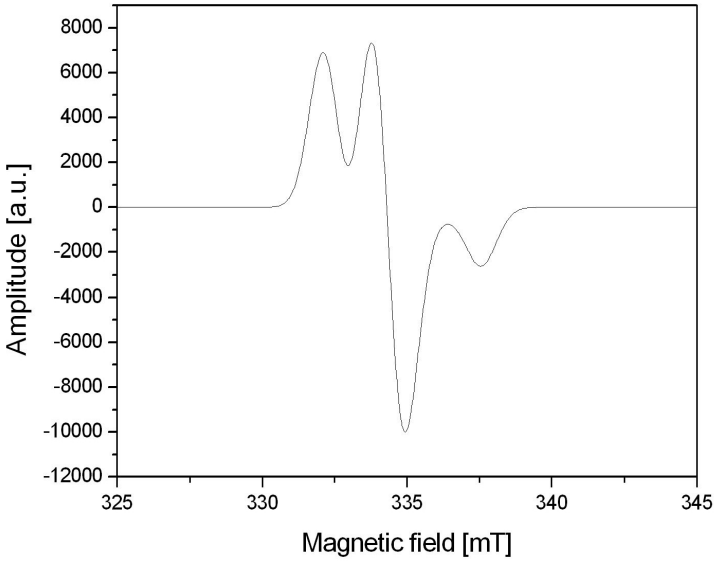

Figure 2. The EPR spectrum simulated in WINEPR SimFonia for following parameters: g1 = 2.01, g2 = 2.0068, g3 = 2.0032, A1 = 0.25 = A2 = 0.25 mT, A3 = 2.68 mT, Width1 = Width2 = Width3 = 1.0 mT

The EPR spectrum was also simulated in WIN EPR SimFonia. The best result (shown in Figure 2), was obtained for the following parameters: g1 = 2.01, g2 = 2.0068, g3 = 2.0032, A1 = A2 = 0.25 mT, A3 = 2.68 mT, Width1 = Width2 = Width3 = 1.0 mT. On the basis of our experimental spectra, on the above simulation and on quantum-mechanical calculations a nitrosobenzene anion radical was described as a radical observed in the human blood-spin trap conglomerate (Figure 3).

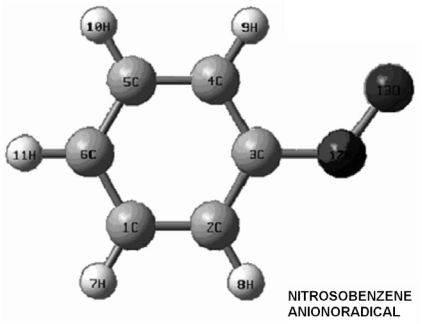

Figure 3. The nitrosobenzene anionoradicals determined in blood-spin trap conglomerate

Samples of the patients' blood were used to determine the following parameters: phosphocreatine kinase (CPK), myoglobin (MB), troponin T (TnT), interleukin-6 (IL6) and ultra-sensitive C-reactive protein (usCRP). These results obtained before, 2 hours and 24 hours after angioplasty and in the control examination are shown in Figures 4 and 5. The values of the investigated parameters increased up to 24 hours after angioplasty, and remained in a normal range 6 months later during control examinations.

Discussion

The shapes of the EPR spectra both before and after angioplasty are similar (Figure 1). However, they differ as to their EPR signal intensities and free radicals concentrations. From three blood samples with a spin trap the biggest signal was observed 2 hours after angioplasty. The growth in the EPR signal intensity is correlated with reperfusion and tissue damage, after which the generation of free radicals is observed [4, 7, 8]. Similar investigations related to free radicals and reperfusion were described in literature [4, 10,

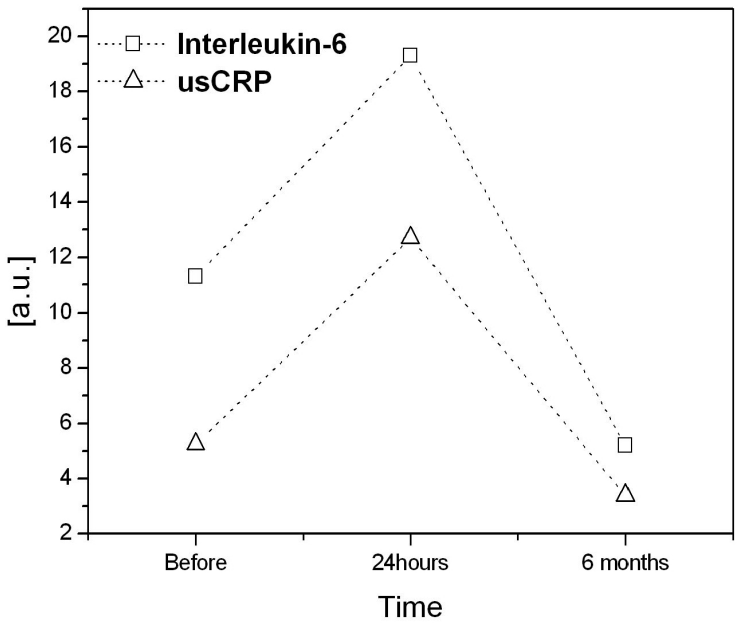

Figure 4. The changes of usCRP and IL-6 values in time of angioplasty

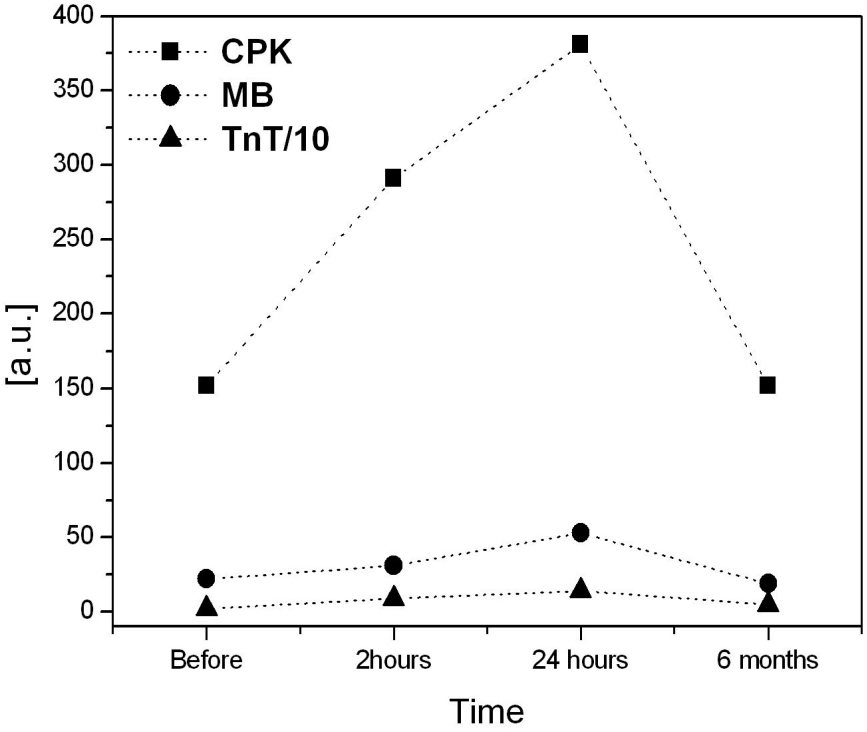

Figure 5. The changes of CPK, MB, TnT values in time of angioplasty

11], however the authors used the tissue of reperfused heart for free radical determination. In our study, the blood with a spin trap was investigated and the results obtained are similar to those reported by Zweier [10, 11]. They also observed increase in EPR signal in a reperfused tissue. In the present study, the concentration of free radicals decreased 24 hours after angioplasty (Figure 1), which probably means that inflammation and damage of the cardiac muscle caused by angioplasty and reperfusion decreases or disappears.

To check the correlation between free radicals and myocardium damage markers, CPK, MB and TnT were determined. The dependence of these parameters on time shows that they increase up to 24 hours after angioplasty (Figures 4 and 5) when the maximum peak is observed, while 6 months later, during control examinations, these values were normal. Medical parameters in angioplasty have been investigated by different scientists and described in literature [5, 6, 9]. Liuzzo [5] observed a similar behaviour for

ultra-sensitive C-reactive protein (usCRP) and interleukin-6 (IL-6) as that in our work (Figure 4). For patients subjected to angioplasty he observed an increase in usCRP and IL-6 for up to 24 hours after angioplasty and then a decrease. These two parameters are good markers of inflammation, which accompanies tissue damage. In the present work, similar results were obtained. The levels of these markers were also measured 6 months later, and the values were normal, which indicates that no inflammation was present.

Similar dependence was observed for myocardium damage markers (Figure 5). The growth in these three parameters (CPK, MB and TnT) was observed about 24 hours after angioplasty. Also in literature [6], the highest peak is observed about 24 hours after reperfusion and then it falls. Some authors claimed that MB could be an early marker of heart damage, and could also be a marker of reperfusion after angioplasty [9]. In our work, the biggest growth in comparison with the primary (before angioplasty) value and 24 hours after angioplasty was observed for the CPK marker (Figure 5).

When the changes in free radical concentration are compared with medical markers it can be stated that all these parameters are good markers of inflammation and tissue damage. However, it seems that free radicals are much more sensitive. It is not possible to use free radical concentration 24 hours after angioplasty (reperfusion) to observe the damage of the cardiac muscle and inflammation. Free radicals are not as stable as medical markers in the blood, and they are probably a starting point for a cascade of changes in the blood.

Conclusions

A nitrosobenzene anion radical was determined as a radical observed in a blood-spin trap conglomerate in patients subjected to angioplasty treatment.

The growth in free radical concentration in the blood-spin trap conglomerate was observed 2 hours after reperfusion.

Twenty four hours after reperfusion the concentration of free radicals decreased and a maximum peak for medical markers was observed.

Free radicals determined by Electron Paramagnetic Resonance is a sensitive method which makes it possible to detect heart damage earlier than using medical markers.

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