PAROXYSMAL ATRIAL FIBRILLATION: DYNAMICS OF THE MAIN ANTIOXIDANT ENZYMES - SUPEROXIDE DISMUTASE AND CATALASE

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ABSTRACT

INTRODUCTION: Researchers have a particularly strong interest in the mechanisms implicated in the clinical manifestation of atrial fibrillation. OBJECTIVE: To examine dynamically the activity of the antioxidant enzymes, superoxide dismutase and catalase in patients with paroxysmal atrial fibrillation (duration < 48 hours). MATERIALS AND METHODS: The studied parameters were examined in the erythrocytes of 51 patients (59.84 ± 1.60, 26 men) immediately after their hospitalization, at 24 hours and 28 days after restoration of sinus rhythm. 52 controls (59.50 ± 1.46, 26 men) were also included, none of which had a history of arrhythmia. Propafenone was used to manage the rhythm abnormality. The enzyme activity was determined by a spectrophotometric method. RESULTS: The average duration of atrial fibrillation episodes until the time of hospitalization was 8.14 hours (from 2 to 24 hours). During patient hospitalization the activity of superoxide dismutase and catalase was considerably higher compared to that of the controls (8.46 ± 0.26 vs 5.81 ± 0.14 U/mg Hb; 7.36 ± 0.25 vs 4.76 ± 0.12 E₂₄₀/min/mg Hb; p < 0.001). This difference was maintained 24 hours after the rhythm regularization (7.19 ± 0.25 vs 5.81 ± 0.14 U/mg Hb, p < 0.001; 5.30 ± 0.21 vs 4.76 ± 0.12 E₂₄₀/min/mg Hb, p < 0.05). Twenty-eight days after the restoration of sinus rhythm, the activity of catalase remained increased (5.11 ± 0.08 vs 4.76 ± 0.12 E₂₄₀/min/mg Hb, p < 0.05). CONCLUSION: The paroxysmal atrial fibrillation in our study was characterized with significantly increased activity of superoxide dismutase and catalase even in the early hours of clinical manifestation of the disorder, which then slowly decreased with the restoration of sinus rhythm. Therefore, we can conclude that changes in oxidative status are closely related to the disease and are probably a part of the intimate mechanisms related to its initiation and clinical course.

Keywords: paroxysmal atrial fibrillation, sinus rhythm, superoxide dismutase, catalase

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ПАРОКСИЗМАЛЬНОЕ ПРЕДСЕРДНОЕ МЕРЦАНИЕ – ДИНАМИКА ОСНОВНЫХ АНТИОКСИДАНТНЫХ ЭНЗИМОВ СУПЕРОКСИД ДИСМУТАЗА И КАТАЛАЗА

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РЕЗЮМЕ

ВВЕДЕНИЕ: Механизмы, связанные с клиническим проявлением предсердного мерцания, вызывают значимый исследовательский интерес. ЦЕЛЬ: Изучить в динамике активность антиоксидантных энзимов супероксид дисмутазы и катаэлаз у пациентов с пароксизмальным предсердным мерцанием давностью < 48 ч. МАТЕРИАЛ И МЕТОДЫ: Показатели исследованы в эритроцитах 51 пациента (59.84 ± 1.60, 26 мужчин) непосредственно после их госпитализации – 24-й час и после восстановления синусового ритма – 28-ой день. Обследовано и 52 здоровых человек в качестве контрольной группы (59.50 ± 1.46, 26 мужчин) у которых не было данных о наличии аритмии. Регулирование ритма у всех пациентов добилось использованием пропафенона. Активность энзимов определена спектрофотометрически. РЕЗУЛЬТАТЫ: Средняя продолжительность предсердного мерцания до момента госпитализации в границах 8.14 ч. – от 2 до 24 ч. При госпитализации пациентов активность супероксид дисмутазы и катаэлаза оказалась значимо более высокой по сравнению с активностью энзимов у контрольной группы (8.46 ± 0.26 vs 5.81 ± 0.14 U/mg Hb; 7.36 ± 0.25 vs 4.76 ± 0.12 E₂₄₀/min/mg Hb; p < 0.001). Разница сохранялась 24 ч. после регуляризации ритма (7.19 ± 0.25 vs 5.81 ± 0.14 U/mg Hb, p < 0.001; 5.30 ± 0.21 vs 4.76 ± 0.12 E₂₄₀/min/mg Hb, p < 0.05) 28 дней после восстановления ритма, активность катаэлазы оставалась значимо выше по сравнению с контрольной группой (5.11 ± 0.08 vs 4.76 ± 0.12 E₂₄₀/min/mg Hb, p < 0.05). ВЫВОДЫ: Эти результаты свидетельствуют о том, что изменения антиоксидантной активности происходят с самого начала заболевания и сохраняются даже в случае отсутствия в клинической картине АФБ, и в дальнейшем могут быть включены в механизм развития и проявления заболевания.
Paroxysmal Atrial Fibrillation: Dynamics of the Main Antioxidant Enzymes - Superoxide Dismutase and Catalase

INTRODUCTION

Atrial fibrillation (AF) is characterized by high morbidity, progressive clinical course and increased risk of thromboembolic complications. AF is defined as an epidemic despite the fact that there are numerous modern treatment modalities for its management. Consequently, the mechanisms associated with its occurrence present a focus of significant research interest.

Over the past years there has been a growing body of evidence indicating a presence of pro-oxidant damage in patients with AF. However, very few are the studies investigating the oxidative status of AF patients during the rhythm disturbance and after sinus rhythm restoration. A study in dynamics could allow researchers to seek a strong correlation between changes in oxidative status and clinical manifestation of AF.

The enzymes superoxide dismutase (SOD) and catalase (CAT) are the first and most important line of antioxidant enzyme defense against pro-oxidant damage. They are involved in eliminating two of the major reactive oxygen species (ROS), namely superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$). Therefore their activity is indicative of the oxidative status of the body. The aim of the present study was to investigate dynamically the antioxidant activity of the enzymes SOD and CAT in patients with paroxysmal atrial fibrillation (PAF) (duration of arrhythmia < 48 hours).

MATERIALS AND METHODS

STUDY PARTICIPANTS

The present study was conducted in the Cardiac Intensive Care Unit of the First Cardiology Clinic at St Marina Hospital in Varna between October 2010 and May 2012 after approval by the Ethics Committee in the hospital and in accordance with the requirements of the Declaration of Helsinki. The participants were recruited in the study after giving their written informed consent.

Only patients with PAF with duration of arrhythmia < 48 hours were screened because this would allow using acute pharmaceutical intervention to restore normal sinus rhythm. Of all 338 patients, 259 dropped out of the study because of the exclusion criteria. The duration of the rhythm disturbance was determined on the basis of a detailed medical history of the patients, in which it was the patient that determined the time of onset of AF by the occurrence of sudden subjective sensation of palpitation that continued until admission to hospital. Electrocardiography was used to confirm the diagnosis.

To restore normal sinus rhythm in the remaining 79 patients, propafenone was given according to the established regimen for administration. There has been no evidence of pro-oxidant or antioxidant effects of this drug reported in the literature. The total duration in the regimen used to restore sinus rhythm with propafenone is 24 hours at the most, and within this time sinus rhythm was restored in 56 participants (31 men, 25 women). By the end of study all patients received propafenone orally in a maintenance dose of 150 mg three times per day. Recurrences of AF were not observed.

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study on middle age people (59.50 ± 1.46 yrs, age range 30-76). The controls had no history of AF, nor did they have any electrocardiographic evidence of AF.

The exclusion criteria included the following diseases and conditions: (1) cardiovascular diseases: coronary heart disease, chronic heart failure, refractory hypertension, implanted device to help control heart rhythm and conduction disorders, inflammatory heart disorders, congenital heart diseases, mild or severe acquired valvular heart disease, cardiomyopathies; (2) other diseases such as kidney or liver failure, diseases of the central nervous system or endocrine system (except for type 2 diabetes mellitus, non-insulin-dependent and well controlled), inflammatory and/or infectious diseases over the last three months, neoplastic or autoimmune disorders, chronic pulmonary disease; (3) hormone replacement therapy, regular reception of analgesics including non-steroidal anti-inflammatory drugs; (4) patients that are unable to detect onset of arrhythmia; (5) persistence of the rhythm disorder after 24-hour propafenone treatment, restoration of sinus rhythm by electrical cardioversion, recurrence of AF by the end of study.

ERYTHROCYTE SAMPLING

Blood samples of patients with PAF were collected three times: immediately after hospitalization at the department (baseline values), at 24 hours and 28 days after restoration of normal sinus rhythm. Choosing day 28 for an end of the observation was done on the basis of a preliminary study. Blood samples from the controls were collected only once.

The activity of SOD and CAT was determined in erythrocytes, derived from 4 ml venous blood collected in heparin vacutainer (Vacuette/4.0 ml/Li HEP). After sampling, the blood was immediately centrifuged at 4° and 600 g for 10 min. The resulting plasma was separated from the erythrocytes, and they were twice washed and rinsed with 1 ml of 0.9% NaCl and centrifuged under the above-described conditions. The resultant suspension of erythrocytes was immediately frozen at -70°C and stored at this temperature up to six months. 5% erythrocyte suspension was used for the examination of the indicators.

METHODS OF MEASURING THE ACTIVITY OF SOD AND CAT

The activity of SOD was determined by the method of Beauchamp & Fridovich. The examined samples were measured spectrophotometrically (560 nm) against blank control (buffer). The inhibition of NBT reduction, caused by superoxide anion, generated from the photoreduction of riboflavin was measured. The activity of the enzyme was expressed as U/mg Hb.

Determination of CAT activity was performed by the method of Aebi. The enzyme containing sample was added to 1.5 ml of 10 mm H₂O₂ (in 50 mM potassium phosphate buffer, pH 7.0) and was measured spectrophotometrically at 240 nm. The activity of the enzyme was indicated against blank reagent (buffer) and was expressed as E₂₄₀/min/mg Hb.

STATISTICAL ANALYSIS

The results were analyzed using GraphPad Prism 4. Descriptive statistics was used to calculate relative percentage, means, standard deviation, standard error of the means and the central tendency (mode). The hypotheses were analyzed using the Student t-criterion to compare means and relative percentage parameters at a level of confidence 0.95 (p < 0.05 were considered statistically significant). The results were presented as means ± standard error of the mean (x ± SEM), as well as data for the standard deviation (SD).

RESULTS

PATIENT CHARACTERISTIC

Table 1 presents the patients and controls characteristics. The patient group was number-, mean age- and gender-matched with the control group (p > 0.05). By indicators such as hypertension, diabetes, dyslipidemia and current treatment, smoking, alcohol drinking, BMI and the measured echographic indicators, the group of PAF patients was statistically identical to the control group (p > 0.05).

As reported in the medical history of patients, the mean duration of AF episodes until hospitalization was 8.14 ± 0.76 hours, and most often the patients often hospitalized 5 hours after onset of arrhythmia (mode = 5 hours; 10 out of all 51 patients). None of the patients was hospitalized after 24 hours.

SOD AND CAT ACTIVITY

Fig. 1 shows the changes in SOD activity, which at baseline (at admission to hospital) was higher that of the controls (8.46 ± 0.26 U/mg Hb, SD = 1.83 vs 5.81 ± 0.14 U/mg Hb, SD = 0.98, p < 0.001). This difference was kept 24 hours after restoration of normal sinus rhythm (7.19 ± 0.25 U/mg Hb, SD = 1.77 vs 5.81 ± 0.14 U/mg Hb, SD = 0.98, p < 0.001). Twenty eight days after discontinuation of arrhythmia, there was no significant difference with the controls (5.90 ± 0.16 U/mg Hb, SD = 1.12 vs
Table 1. Characteristics of PAF patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients with PAF</th>
<th>Control Group</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants in the group</td>
<td>51</td>
<td>52</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.84 ± 1.60</td>
<td>59.50 ± 1.46</td>
<td>0.16</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Men/Women</td>
<td>26/25</td>
<td>26/26</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Cardiovascular disease risk factors

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Patients with PAF</th>
<th>Control Group</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>37 (72.5%)</td>
<td>34 (65.4%)</td>
<td>0.78</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Diabetes mellitus type 2</td>
<td>3 (5.9%)</td>
<td>2 (3.8%)</td>
<td>0.50</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>4 (7.8%)</td>
<td>3 (5.8%)</td>
<td>0.40</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Smoking*</td>
<td>8 (15.7%)</td>
<td>7 (13.5%)</td>
<td>0.31</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Drinking alcohol**</td>
<td>7 (13.7%)</td>
<td>6 (11.5%)</td>
<td>0.34</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.85 ± 0.46</td>
<td>24.95 ± 0.45</td>
<td>1.69</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>19 (37.3%)</td>
<td>17 (32.7%)</td>
<td>0.51</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>15 (29.4%)</td>
<td>14 (26.9%)</td>
<td>0.28</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Sartans</td>
<td>11 (21.6%)</td>
<td>9 (17.3%)</td>
<td>0.55</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Statins</td>
<td>4 (7.8%)</td>
<td>3 (5.8%)</td>
<td>0.40</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>LVEDD mm</td>
<td>52.57 ± 0.58</td>
<td>52.29 ± 0.57</td>
<td>0.34</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>LVEF%</td>
<td>62.98 ± 0.70</td>
<td>61.54 ± 0.58</td>
<td>1.57</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>LA volume ml/m²</td>
<td>22.81 ± 0.45</td>
<td>23.82 ± 0.48</td>
<td>1.53</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

LA - left atrium; LVEF - left ventricular ejection fraction; LVEDD - left ventricular end-diastolic diameter; (The data are presented as x ± SEM).

* No more than half a box of cigarettes a week. Hospitalized patients had not smoked at least 24-48 hours before onset of arrhythmia. The lab tests for the controls, and those on day 28 after discharge of patients were done after a 48-hour non-smoking interval.

** No more than 1-2 drinks/week. Hospitalized patients had not drunk alcohol at least 48 hours before onset of arrhythmia. The lab tests for the controls, and those on day 28 after discharge of patients were done after a 48-hour non-drinking interval.

5.81 ± 0.14 U/mg Hb, SD = 0.98, p > 0.05).

Fig. 2 shows that CAT activity at baseline was significantly higher than that of the controls (7.36 ± 0.25 E240/min/mg Hb, SD = 1.76 vs 4.76 ± 0.12 E240/min/mg Hb, SD = 0.87, p < 0.001). The activity of the enzyme measured at 24 hours and 28 days after normal rhythm restoration was again higher that of the controls (5.30 ± 0.21 E240/min/mg Hb, SD = 1.53 vs 4.76 ± 0.12 E240/min/mg Hb, SD = 0.87, p < 0.05 and 5.11 ± 0.08 E240/min/mg Hb, SD = 0.56 vs 4.76 ± 0.12 E240/min/mg Hb, SD = 0.87, p < 0.05, respectively).

**DISCUSSION**

The enzyme activity of SOD and CAT was significantly increased at hospitalization of patients (Figs 1, 2). The enzyme superoxide dismutase is commonly known as the main defender of the body against superoxide anion (O2–) and catalyses its dismutation to H2O2. It is, in its turn, neutralized by CAT by converting it to water and oxygen.14 This is the reason why the activity of SOD and CAT is of crucial importance for the elimination of O2– and H2O2. Consequently, we can safely assume that the enzymatic changes we found are an adaptive response to the increased levels of O2– and H2O2.

The superoxide anion is the initial reactive oxygen species (ROS), which makes its dismutation by SOD to be of primary importance for every cell.15,16 This anion is formed after a one electron
transfer to oxygen. It has a relatively short half-life and may transfer into a number of other ROS such as H₂O₂, peroxynitrite or hydroxyl anions, etc.¹⁷ If these have high concentrations, they can readily react with lipids, proteins and DNA molecules and damage their structures. All this causes cell damages and oxidative stress.¹⁷ It is difficult to determine directly the levels of free radicals because of their short half-life. Therefore, an assessment of the antioxidant defense system is a good indicator in searching oxidative stress and the increased activity of SOD and CAT is an important indirect indication of increased pro-oxidant damage.⁷,¹⁸

These data reported in the literature allow us to conclude that the increased levels of SOD and CAT we found in this study indicate a disrupted oxidative status in patients with PAF. Furthermore, the disruption was detected very soon after beginning of arrhythmia (8.14 hours on average), which is a serious argument about a very strong correlation between them and the clinical manifestation of the disease.

Twenty-four hours after restoration of normal sinus rhythm, the activity of SOD and CAT, judged by its absolute values, started to decline quite distinctly but was still considerably higher.

** - p < 0.001; NS - not significant difference; Data are presented as x ± SEM.

** Figure 1. Dynamics in SOD activity (U/mg Hb) in PAF patients erythrocytes.

** Figure 2. Dynamics of CAT activity (E₂₄₀/min/mg Hb) in PAF patients’ erythrocytes.
than that in the control group. These results are clearly indicative of increased production of ROS even after the arrhythmia episode.

At day 28 there was still a downward trend to normalization of the studied parameters and the values for SOD activity did not differ any longer from those in the controls, while CAT activity was still high. The restoration of the sinus rhythm is associated with a reduction of the oxidative stress.

Studying the antioxidant system in dynamics shows that the increased levels of ROS in patients with PAF and, therefore, the consequences that are a result of them, are not incidental in the clinical course of arrhythmia.

It is known that ROS are implicated in the modulation of mitogen-activated protein kinase subfamilies such as extracellular signal-regulated kinase, c-Jun N-terminal kinase and p38 kinase. These, in their turn, induce the development of atrial fibrosis. This slows down the conduction of electrical impulses and enables the occurrence of re-entry mechanisms of excitation in the atrial myocardium and the initiation of AF. Therefore, rapid cessation of arrhythmia and an effective anti-relapsing therapy would make it possible to break the vicious circle of the self-sustaining atrial fibrillation.

**CONCLUSIONS**

In patients with PAF, the activity of the enzymes SOD and CAT is significantly increased even in the early hours of clinical manifestation of the disorder and slowly decreases after restoration of the sinus rhythm. This specific dynamics allows us to conclude that the changes in the oxidative status are closely related to the disease and are probably a part of the intimate initiation mechanisms and the clinical course. Moreover, these results clearly argue for an antioxidant treatment to become part of the therapeutic strategy in atrial fibrillation.

**REFERENCES**