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Red-ox reactions and its serum markers in patients with pulmonary embolism

Reakcje *red-ox* i ich markery w surowicy krwi u pacjentów z zatorem tętnicy płucnej

INTRODUCTION

Pulmonary embolism involves the activation of blood platelets and neutrophils and generation of toxic oxygen products by these cells [1, 2, 4, 8, 9]. Oxygen radicals generated close to a cell membrane oxidise membrane phospholipids (lipid peroxidation), a process which may continue in a chain reaction [1, 2, 4, 8]. A major site of free radical attack is on polyunsaturated fatty acids in cell membranes, producing lipid peroxidation which generates hydroperoxides and long lived aldehydes [1, 3, 6]. The end products of these reactions are conjugated dienes (CD) and malonaldehyde (MDA). This pathophysiologiv process is directly responsible for injury of lung vascular endothelilal cells [1, 4, 6, 8] Glutathione peroxidase (GSH-Px) plays an important role in protection against oxidative damage of lung [2, 3, 5, 8]. We hypothesied, that lung injury in patients with pulmonary embolism, mediated by oxygen free radicals can be monitored by the apperance in plasma of lipid peroxidation products like conjugated dienes (CD) and malonaldehyde (MDA).

MATERIALS AND METHODS

We analyzed 25 patients (17 male, 8 female) aged from 30 to 70, with pulmonary embolism as a complication of the long bones or hip fracture. Venous blood was obtained by venipuncture from study patients before thrombolytic treatment and after 1, 2, 3 days of treatment. All patients received heparin intravenously (30,000 U.j. per

day) durig study period and later, as long as needed. In the same time we obtained blood sample from healthy voluneers as control group. Platelets and neutrophils counts were determined with routine methods. We measured generation of toxic oxygen products by zymozan-stimulated neutrophils and thrombin-stimulated platelets *in vitro*.

Plasma levels of lipid peroxidation products like conjugated dienes (characteristic absorbance 233 nm) and malonaldehyde (characteristic absorbance 535 nm) were evaluated by colorimetric determinations [6, 7]. Oxygen free radicals generation by zymozan- stimulated neutrophils and thrombin-stimulated platelets were evaluated in nM/min/1mln cells in supernatants [6, 7].

Glutathione peroxidase released by platelets and neutrophils were measured using spectrophotometry in mUx 10.9 cells, in supernatants. For determination of statistically significance of the data, the t-test was employed.

RESULTS AND DISCUSSION

The highest generation of oxygen free radicals by zymozan-stimulated neutrophils (mean value 81 nM/min/1 mln cells, control mean value 38 nM/min/1 mln cells) and thrombin-stimulated platelets (mean value 57 nM/min/ 1mln cells, control mean value 23 nM/min/1 mln cells) before thrombolytic treatment was observed, ($p < 0.05$). High plasma levels of lipid peroxidation products like conjugated dienes (mean values were from 1.1 to 1.4 nM/ml, 233 nm absorbance, control mean value 0.07 nM/ml) showed close correlation with the appearance in plasma levels of malonaldehyde (mean values were from 0.08 to 0.1 nM/ml, 536 absorbance, control mean value was 0.02 nM/ml) during the whole study period ($p < 0.05$).

No statistically significant differences in glutathione peroxidase activity were found between control and study group. In the light of the recent studies neutrophils are key participants in the development if tissue injury in a variety diseases including pulmonary embolism [4, 6, 7, 8, 10]. It has been proposed that neutrophil recruitment to sites of tissue in several steps involving: rolling of the cells on the activated endothelium of the blood vessel wall, activation of neutrophils, adhesion of neutrophils to the endothelium and in the end migration of neutrophils into the surrounding tissue [4, 6, 8, 10]. Tissue injury develops rapidly and is known to be dependent on toxic oxygen products generated from neutrophils. Platelets have been implicated as mediators in inflammatory responses of vessel walls platelet-neutrophil interactions and oxidative tissue damage [4, 5, 8, 9]. Platelets stimulated by thrombin also augmented of the free radicals production of neutrophils. Both stimulated and unstimulated platelets enhanced the free radicals release induced by zymozan-stimulated neutrophils. Platelet activating factor (PAF) may be generated both in platelets and neutrophils and is also a potent neutrophil stimulus [9, 10]. To investigate whether neutrophils respond to platelets in thromboembolic disease, we applied a method that rendered it possible to monitor the activity of both cell types simultaneously in the same sample. During the whole study period we observed low activity of glutathione peroxidase as an indicator of antioxidative defence of lung. This low activity of GSH-Px was probably associated

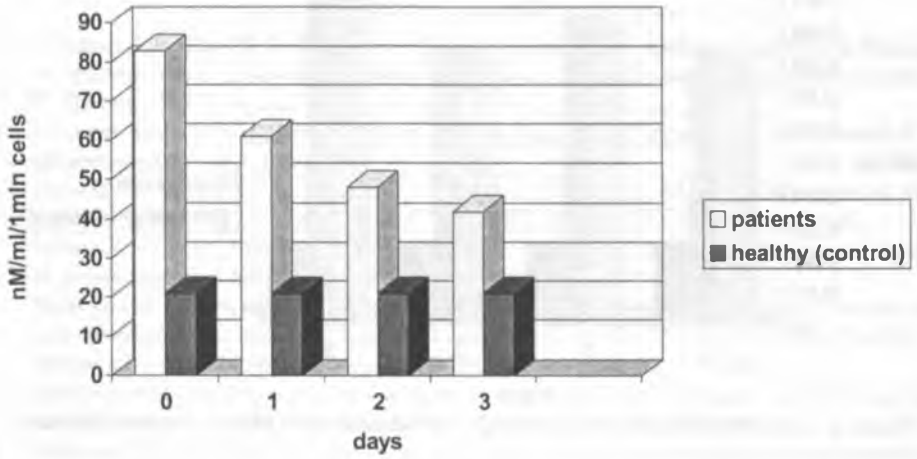

Figure 1. Oxygen free radicals release by zymozan-stimulated neutrophils

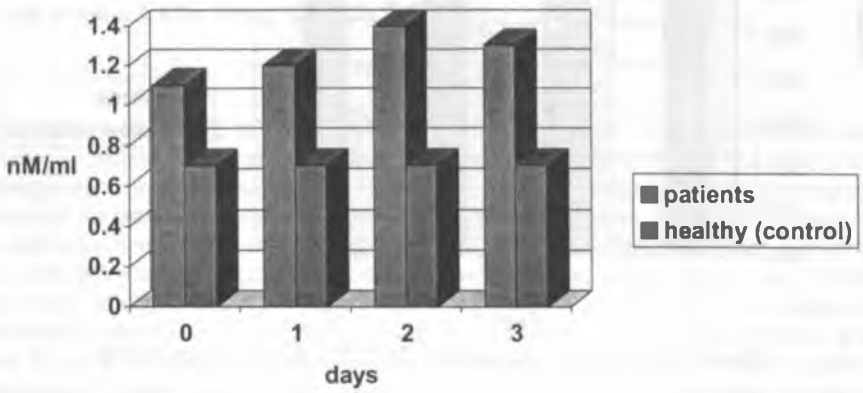

Figure 2. Lipid peroxidation products plasma conjugated dienes (233nm)

with an increased production of reactive oxygen radicals, which may inactivate this enzyme [2, 3, 5, 8].

CONCLUSIONS

The data presented in our study suggest, that lung injury in patients with pulmonary embolism may be associated with the appearance various products of lipid peroxidation. It is possible, that lipis peroxidation products like conjugated dienes and malonaldehyde may be useful markers of oxygen-related lung injury in thromboembolic disease.

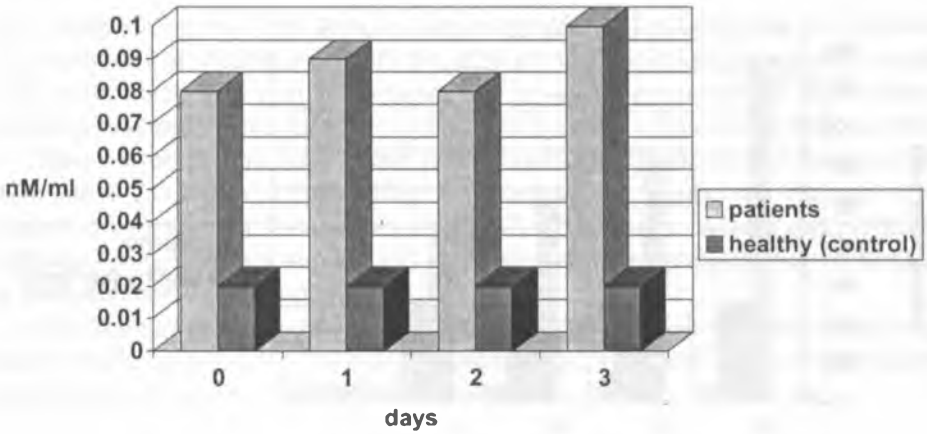

Figure 3. Lipid peroxidation products plasma malonylaldehyd (MDA) contents (535 nm)

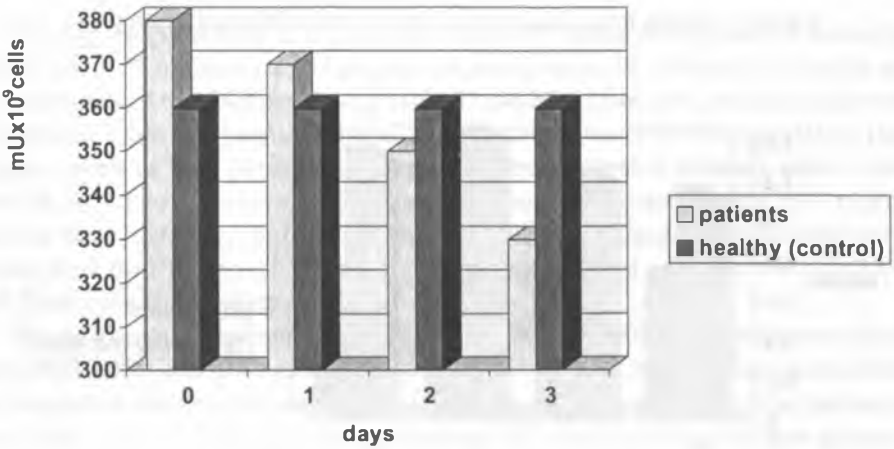

Figure 4. Glutathione peroxidase (GSH-Px) release by neutrophils

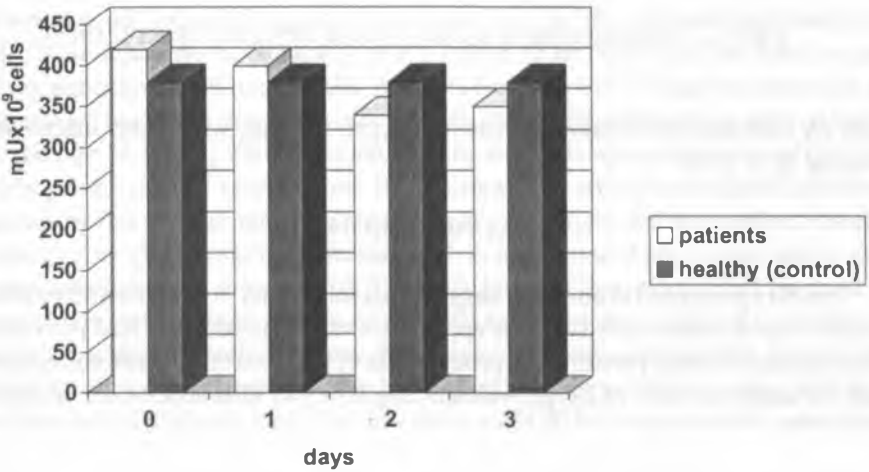

Figure 5. Glutatione peroxidase (GSH-Px) release by thrombin stimulated platelets

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STRESZCZENIE

Zachwianie równowagi między mechanizmami oksydacyjnymi i antyoksydacyjnymi leży u podstaw patogenezy oksydacyjnego uszkodzenia płuc. Zator tętnicy płucnej jest procesem patofizjologicznym, w którym dochodzi do aktywacji płytek krwi i neutrofili oraz wydzielania przez te komórki toksycznych produktów przemiany tlenowej. Głównym przedmiotem ataku wolnych rodników tlenowych są wielonienasycone kwasy tłuszczowe obecne w błonach komórkowych, z których w wyniku procesów peroksydacyjnych uwalniają się sprzężone dieny (CD) oraz długo żyjące aldehydy (MDA). Poważną rolę w ochronie antyoksydacyjnej płuc odgrywa peroksydaza glutationu (GSH-Px). Obserwowaliśmy 25 pacjentów (17 mężczyzn, 8 kobiet) w wieku od 30 do 70 lat, z zatorem tętnicy płucnej. 13 pacjentów miało zakrzepicę żył głębokich potwierdzoną ultrasonografią typu Doppler, 12 pacjentów miało zator tętnicy płucnej jako powikłanie złamania kości długich lub biodra. Krew do badania pobierano od osób zdrowych oraz badanych pacjentów przez nakłucie żyły przed leczeniem oraz po 1, 2, 3 dniach leczenia. Liczbę płytek krwi oraz neutrofili oznaczano według rutynowych metod. Produkcję toksycznych produktów tlenowych przez stymulowane zymozanem neutrofile oraz stymulowane trombiną płytki krwi oznaczano w warunkach *in vitro*. Poziom CD oraz MDA w surowicy krwi oceniano przy użyciu metod kolorymetrycznych. Aktywność GSH-Px oceniano przy użyciu metod spektrofotometrycznych. W grupie badanej najwyższą produkcję wolnych rodników tlenowych obserwowano w okresie przed rozpoczęciem leczenia (dla płytek: wartość średnia 57 nM/min/1 mln komórek, kontrola: wartość średnia 38 nM/min/1 mln komórek) Nie stwierdzono istotnych statystycznie różnic aktywności GSH-Px miedzy grupą badaną i kontrolną. Stężenie CD w surowicy krwi (wartość średnia:1,3 nM/ml, kontrola: wartość średnia: 0,07 nM/ml, $p < 0,05$) ściśle korelowało z obecnością w surowicy krwi MDA (wartość średnia: 0,1 nM/ml, kontrola: wartość średnia 0,02 nM/ml, $p < 0,05$) w czasie całego okresu obserwacji. Dane prezentowane w badaniu sugerują, że uszkodzenie płuc u pacjentów z zatorem tętnicy płucnej może być związane z obecnością w surowicy krwi wysokich stężeń toksycznych produktów peroksydacji lipidów błon komórkowych takich jak MDA i CD.