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*Oxidative metabolism of neutrophils in patients with initial
„outside-hospital” pneumonia*

The respiratory system is in continuous contact with the changing environmental factors. This contact is likely to result in various diseases, particularly in the development of defensive mechanisms in the infected pulmonary tissue pneumonia. Despite numerous studies, the role, function and course of the successive stages of pneumonia have not been fully explained yet.

The systemic circulation neutrophils remain unstimulated; due to the inflammatory reaction resulting from chemotactic, mobilizing and activating abilities, they become the main defensive line of the organism against pathogens, apart from macrophages. Their activation leads to the formation and release of many toxic substances whose aim is to destroy the invasive particles. After reaching the infection focus, the neutrophils may destroy pathogens in two different ways: by the aerobic or anaerobic mechanism.

The purpose of the study was to assess the oxidative metabolism of the unstimulated and zymosan-stimulated neutrophils expressed as the production of superoxide anion radicals and to determine the plasma O₂ levels in the patients with initial “outside-hospital” pneumonia.

MATERIAL AND METHODS

The study included 20 patients with the initial symptoms of “outside-hospital” pneumonia. The patients’ age ranged from 26 to 70 years (average – 49). The control group consisted of 18 healthy volunteers aged 18-59 (average – 42). The patients had clinical symptoms of “outside-hospital” pneumonia (dyspnoea, cough, elevated temperature, typical auscultatory changes) confirmed by the chest X-ray. The patients with coexisting chronic diseases and those who started their antibiotic treatment at home were excluded. The plasma erythrocytes and leukocytes were isolated from the basilic vein blood collected in fasting state, and the preparation containing over 99% of neutrophils was prepared. The amount of superoxide anion radicals produced by neutrophils and their plasma levels were determined using the method of cytochrome reduction – c 5×10^{-5} M/l. Moreover, the measurements in the zymosan-stimulated granulocytes (Sigma, St.Luis, USA) opsonized in the autologous serum according to Markert et al. and prepared according to Weiss were performed.

RESULTS

Table 1

	Control group (n=18)	Examined group (n=20)
O ₂ production (nM/10 ⁶ PMNL) in the unstimulated neutrophils	1.7±3.4	19.8±3.4*
O ₂ production(nM/10 ⁶ PMNL) in the zymosan-stimulated neutrophils	31.4±4.7	44.2±4.1*
O ₂ concentration (nM of reduced cytochrome c/min/mg of protein)	0.1±0.025	0.28±0.03*

± S D, *p0<.05

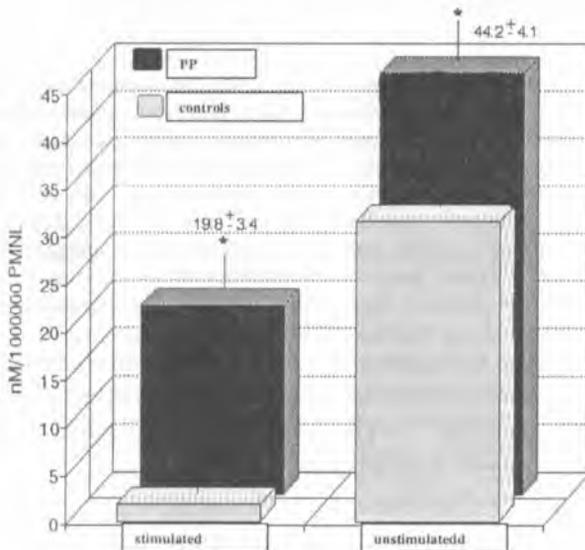


Fig.1. Production of superoxide anion radicals (O₂) by the unstimulated neutrophils and in response to the 30-minute zymosan stimulation. Average values ± SD, * p<0.05 pp vs c, pp-pneumonia patients, c- controls

The data presented in the figure show that the unstimulated neutrophils in the pneumonia patients produced almost 12 times higher amount of O₂ than those in the controls, in absolute values – 19.8±3.4 and 1.7±3.4 nM/1000000PMNL, respectively. These differences were statistically significant. However, after the 30-minute zymosan stimulation, the pneumonia patients` neutrophils produced only 1.4 times higher amount of O₂ than those of healthy individuals – 44.2±4.1 and 31.4±4.7 nM/1000000 PMN, respectively. The differences were also statistically significant.

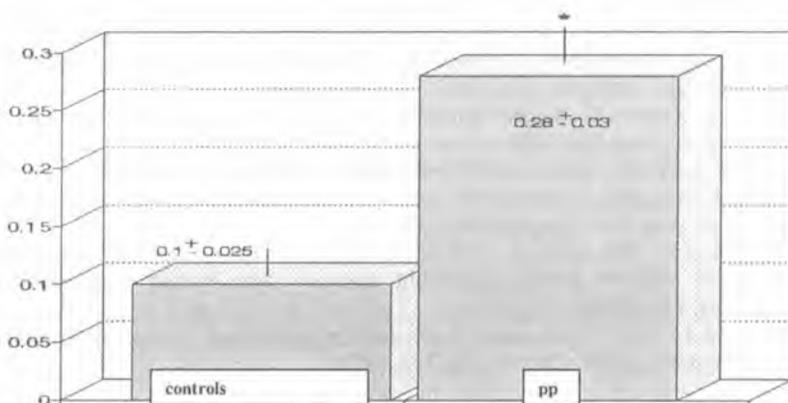


Fig. 2. The plasma concentration of superoxide anion radicals (O_2) in both groups expressed in nM of reduced cytochrome c/min/mg of protein. The average values \pm S.D. * $p < 0.05$ pp vs c, pp – pneumonia patients, c – controls

Compared to the control group, in which the O_2 level was 0.1 ± 0.025 nM of reduced cytochrome c/min/mg of protein, the plasma level of free superoxide radicals in the pneumonia patients was statistically significantly higher – 0.28 ± 0.030 nM of reduced cytochrome c/min/mg of protein.

The findings reveal that the neutrophils and free radical substances produced by them play an important role in the pathogenesis and course of pneumonia in humans. The active oxygen compounds generated by the stimulated neutrophils are relevant bacteriocidal factors, their excessive release is likely to result in further pathologies.

It seems necessary to continue the studies concerning the possibilities of affecting the metabolism of neutrophils in pneumonia by using the specific drugs which would provide the maximum defensive use of neutrophils and prevent their aggression.

CONCLUSIONS

1. Patients with initial pneumonia show increased oxidative metabolism demonstrated by higher production of superoxide anion radicals by neutrophils and by elevated plasma O_2 levels.

2. Determinations of the plasma superoxide anion radical levels in pneumonia patients may be a practical and easily accessible indicator of the activation of neutrophil oxidative metabolism.

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SUMMARY

The aim of the study was to assess the oxidative metabolism of the unstimulated and zymosan-stimulated neutrophils expressed as the production of superoxide anion radicals, and to determine the plasma O_2 levels in the patients with initial "outside-hospital" pneumonia. The study included 20 patients with the initial symptoms of "outside-hospital" pneumonia. The plasma erythrocytes and leukocytes were isolated from the basilic vein blood collected in fasting state, and the preparation containing over 99% of neutrophils was prepared. The amount of superoxide anion radicals produced by neutrophils and their plasma levels were determined using the method of cytochrome reduction – c 5×10^{-5} M/l. The patients with initial pneumonia show increased oxidative metabolism demonstrated by higher production of superoxide anion radicals by neutrophils and by elevated plasma O_2 levels. Determinations of the plasma superoxide anion radical levels in pneumonia patients may be a practical and easily accessible indicator of the activation of neutrophil oxidative metabolism.

Metabolizm tlenowy granulocytów obojętnochłonnych u chorych w początkowym okresie pozaszpitalnego zapalenia płuc

Celem pracy była ocena wpływu egzogennych zmiataczy wolnych rodników na produkcję trwałych utleniaczy w stymulowanych zymosanem granulocytach obojętnochłonnych u chorych w początkowym okresie pozaszpitalnego zapalenia płuc. Badaniem objęto 20 chorych z początkowymi objawami „pozaszpitalnego” zapalenia płuc. Grupę kontrolną stanowiło 18 zdrowych ochotników. Celem oceny egzogennych „zmiataczy” wolnych rodników w stymulowanych zymosanem granulocytach obojętnochłonnych inkubowano je w obecności dysmutazy nadtlenkowej, benzoenu sodu, mannitolu lub dwumetylosulfotlenku. Otrzymanego bezkomórkowego supernatantu używano do oznaczeń spektrofotometrycznych. Badanie wykazało, że zmiatacze „wolnych rodników” zmniejszają produkcję trwałych utleniaczy przez granulocyty obojętnochłonne u chorych z zapaleniem płuc. Zastosowanie leków o właściwościach zmiataczy „wolnych rodników” w leczeniu wspomagającym może w znaczący sposób ograniczyć niszczenie tkanki płucnej, powodowane przez nadmiernie pobudzone w przebiegu procesu zapalnego neutrofile.