

# Effects of Low Intensity Magnetic Fields and Red Light on Respiratory Burst of Neutrophils

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## Abstract

The effect of the magnetic field with red light (PMiR) generated by a special generator- Viofor JPS in the distance of 1cm upon the extramitochondrial respiratory burst of human neutrophils in vitro has been investigated. Blood samples from blood donors were used for the study. DCFH-DA was used for estimation of the respiratory burst, with PMA (phorbol 12-myristate, 13-acetate) as activator of neutrophils. Fluorescence was measured by use of flow cytometry. A decrease of reactive oxygen species production by neutrophils stimulated with PMA was observed. The effect was statistically significant. This influence was detectable at the intensity of 44,5  $\mu$ T. The difference in respiratory burst between native samples and those influenced by PMiR was statistically significant when the intensity of magnetic field was also 44,5  $\mu$ T and the mean of power of light was 40,3 mW. Every statistically significant influence was observed only for stimulated neutrophils when the intensity was 44,5  $\mu$ T. This let us assume that the "window effect" exists.

**Keywords:** magnetic field, respiratory burst, neutrophils

## Introduction

Magnetostimulation is an effect on an organism with a weak slow-changing electromagnetic field (less than 100  $\mu$ T), which brings about a functional equilibrium (homeostasis). It is a safe method, useful in the prophylaxis as well as a supplement to primary treatment. We have investigated whether the magnetostimulation with red light can influence the human neutrophil function in vitro. Other laboratories carried out researches on the influence of magnetic field or infrared or red radiation on neutrophils [1]. Neutrophils are highly specialized white blood cells, contributing to the immune response. Basic function of neutrophils is executing the phagocytosis and successive killing of phagocytized microorganisms. Neutrophils possess several intercellular mechanisms of killing pathogens e.g. the reactive oxygen species production (ROS), called oxidative burst. Within steady-state cells, the

ROS production is relatively low, and therefore the stimulation of cells with various substances, e.g. with PMA is applied on in vitro cultures [2].

## Experimental procedures

For the purpose of the research, the blood from healthy volunteers was taken on anticoagulant. The examined samples were incubated in alternating magnetic field in the range of ELF, combined with the red light (PMiR), on three different induction levels for 30 minutes. The induced respiratory burst was assessed by the intracellular oxidative transformation of DCFH-DA (2',7'-dichlorofluorescein diacetate) to the fluorescent DCF (2',7'-dichlorofluorescein) via cytometry flow. The respiratory burst was induced with the PMA (phorbol 12-myristate, 13-acetate), to produce submaximal stimulation of the respiratory burst [2]. The

kind of magnetic field used was type  $M_1P_3$ , which was generated by a special generator Viofor JPS. In this program the duration of ionic cyclotron resonance effect ( $t_{ICR}$ ) is longer than the sum of the duration of electrodynamic ( $t_{ED}$ ) and magnetomechanic effect ( $t_{MG}$ ) (equation 1). ICR effect on human body is non-linear.

$$\frac{t_{ICR}}{t_{ED} + t_{MG}} \gg 1 \quad (1)$$

where:

- $t_{ICR}$  – duration of ionic cyclotron resonance effect (ICR),
- $t_{ED}$  – duration of electrodynamic effect (ED),
- $t_{MG}$  – duration of magnetomechanic effect (MG)

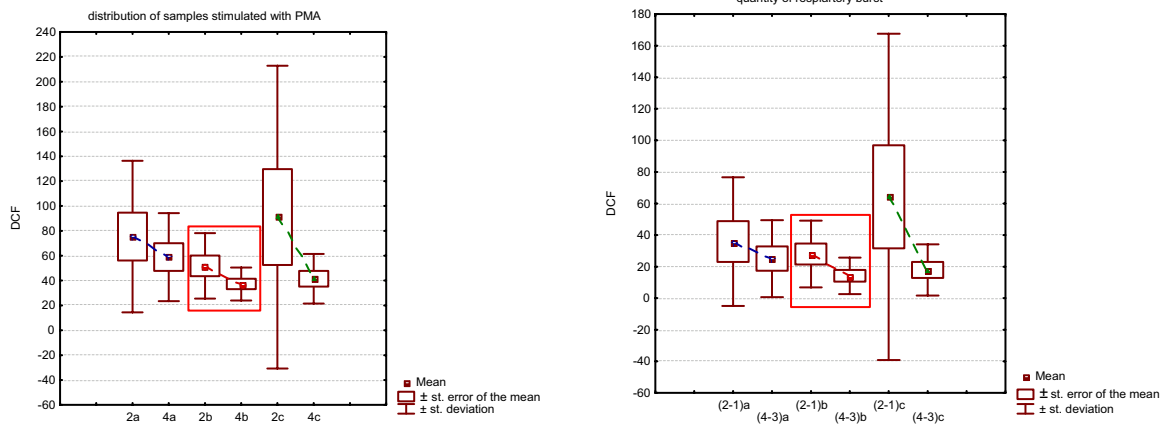
The power density in a single impulse of red light was  $10,7 \text{ mW/cm}^2$ . The average power of red light for

application  $M_1P_3$  was  $30,2 \text{ mW}$  when  $B_1=26,7 \text{ }\mu\text{T}$  and  $40,3 \text{ mW}$  when  $B_2=44,5 \text{ }\mu\text{T}$  or  $B_3=89 \text{ }\mu\text{T}$ . The source (an applicator) of magnetic field combined with red light was placed on the distance of  $1 \text{ cm}$  below the examined samples.

For statistical analysis, the Wilcoxon test, K-S test and descriptive statistic were used.

## Results

The influence of PMiR on respiratory burst is presented in Table 1. We observed a reduction of the ROS production by neutrophils for all used applications. This effect was statistically significant (Table 1) only at the intensity of  $44,5 \text{ }\mu\text{T}$  for PMA stimulated neutrophils. We observed that the production of reactive oxygen species was statistically significant (Table 1) between neutrophils under the



**Fig. 1.** Distribution of blood samples stimulated with PMA (a) and Quantity of respiratory burst (b). When “a” means  $B=26,7 \text{ }\mu\text{T}$ , “b” means  $B=44,5 \text{ }\mu\text{T}$  and “c”  $B=89 \text{ }\mu\text{T}$ . The window marks statistically significant changes. The blood samples have been marked as follows: 1- a sample of blood unstimulated with PMA, without the influence PMiR, 2- a sample of blood stimulated with PMA, without the influence PMiR, 3- a sample of blood unstimulated with PMA, under the influence PMiR, 4- a sample of blood stimulated with PMA, under the influence PMiR. Below, there are also the marks of the difference in fluorescence intensity of DCF values: (4-3) – respiratory burst in samples influenced by PMiR, (2-1) – respiratory burst in native samples

**Table 1.** Values of DCF fluorescence and significant level p obtained from Wilcoxon test for all samples.

samples	$B_1=26,7 \text{ }\mu\text{T}$		$B_2=44,5 \text{ }\mu\text{T}$		$B_3=89 \text{ }\mu\text{T}$	
	mean	st. dev.	mean	st. dev.	mean	st. dev.
1	39,5	30,9	23,9	10,0	26,9	19,1
2	75,4	61,0	51,9	26,3	91,1	121,8
3	33,8	21,2	23,1	8,8	23,5	12,6
4	58,9	35,3	37,3	13,3	41,5	19,9
	significant level p					
1&3	0,96		0,44		0,96	
2&4	0,33		0,02		0,09	
(4-3) & (2-1)	0,28		0,04		0,11	

influence PMiR (4-3) and neutrophils without the influence of PMiR (2-1). This value described the quantity of the respiratory burst. The magnetic field connected with the red light decreases the ROS production by neutrophils. Fig. 1a illustrates the distribution of samples stimulated with PMA for all used application and Fig. 1b illustrates the quantity of respiratory burst in samples influenced by PMiR (4-3) and in native samples (2-1). We can observe the distribution of samples when  $B=44,5 \mu\text{T}$  is narrower than the other application. There are trends of the reactive oxygen species production decrease.

### Discussion

This research shows us the influence of the magnetic field connected with the red light on the oxygen metabolism of neutrophils. A statistically significant decrease of reactive oxygen species production by neutrophils stimulated with PMA was observed, as shown in the results achieved by E. A. Sheiko [3]. This influence was detectable only at the intensity of  $44,5 \mu\text{T}$ , and only for stimulated neutrophils. The difference in respiratory burst between native samples and those influenced by PMiR was statistically significant, when the same intensity ( $44,5 \mu\text{T}$ ) was applied. This phenomenon is called the “amplitude

window effect”, which was also observed in the other researches [4]. We believe that continuation of the research will allow characterizing the width of the window for factors such as the magnetic field (magnetostimulation), the red light and the magnetic field connected with the red light, which influence oxygen metabolism of neutrophils.

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