

Effect of magnetic resonance imaging on human respiratory burst of neutrophils

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Abstract It is known that low intensity magnetic fields increase superoxide anion production during the respiratory burst of rat peritoneal neutrophils *in vitro*. We investigated whether the high intensity magnetic fields (1.5 T) during magnetic resonance imaging can influence the human neutrophil function under *in vivo* conditions. Blood samples were obtained from 12 patients immediately before and after magnetic resonance imaging (mean time 27.6(±11.4 min)). The induced respiratory burst was investigated by the intracellular oxidative transformation of dihydrorhodamine 123 to the fluorescent dye rhodamine 123 via flow cytometry. The respiratory burst was induced either with phorbol 12-myristate 13-acetate, *Escherichia coli*, *N*-formyl-methionyl-leucylphenylalanine or priming with tumor necrosis factor followed by FMLP stimulation. There was no significant difference between the respiratory burst before and after magnetic resonance imaging, irrespective of the stimulating agent. Short time exposure to a high intensity magnetic field during magnetic resonance imaging seems not to influence the production of radical species in living neutrophils.

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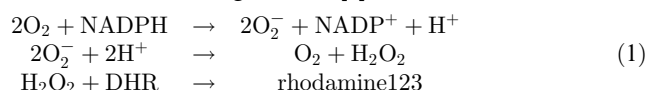
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1. Introduction

It has been assumed that frequency as well as static magnetic fields (MF) can increase free radical concentrations in biological systems [1]. An experimental observation showed that a low frequency (60 Hz), low intensity (0.1 mT) MF increases free radical species in living cells [2]. The authors investigated the phorbol 12-myristate 13-acetate (PMA) induced superoxide anion production during the respiratory burst (RB) in primed rat peritoneal neutrophils. The amount of free radical-derived oxidants after stimulation by PMA was detected by formation of the highly fluorescent compound 2',7'-dichlorofluorescein (DCF) with a monochromator spectrophotofluorimeter. They found a 12.4% increase in the DCF fluorescence signal in the presence of low frequency, low intensity MF [2]. The aim of our study was to investigate if short time exposure (about 30 min) to high intensity static MF (1.5 T) of a magnetic resonance tomograph induces similar effects on the RB of neutrophils in patients undergoing magnetic resonance imaging (MRI).

In our study the neutrophil RB was assayed with a multi-

parameter flow cytometry technique using dihydrorhodamine 123 (DHR) [3]. Oxidation of DHR occurs after the RB following treatment of neutrophils with certain stimulation agents. The assay depends upon the incorporation of DHR into the cell. After cell activation, NADPH oxidase catalyzes the reduction of oxygen to the superoxide anion, which is further produced by dismutation to hydrogen peroxide (H₂O₂). The non-fluorescent DHR is oxidized intracellularly in a peroxidase-dependent reaction to green fluorescent rhodamine 123. The green fluorescence produced is proportional to the amount of H₂O₂ generated [3]:



In our study the RB was induced from three different sources: direct stimulation of protein kinase C by means of PMA, phagocytosis of bacteria (*Escherichia coli*), stimulation with a bacterial peptide (*N*-formyl-methionyl-leucylphenylalanine, FMLP) and by priming with tumor necrosis factor alpha (TNF-α) followed by FMLP stimulation. The quantities of PMA, *E. coli*, FMLP and TNF-α/FMLP were titrated to produce submaximal stimulation of the RB. Therefore, potential suppression or augmentation of the RB by MRI could be proved simultaneously.

2. Materials and methods

After the ethical committee's approval and informed consent, blood samples from 12 patients (six male, six female, aged 31–64 years, mean = 46.7 years) undergoing MRI, were investigated at the Neuro-radiological Department of the Hannover Medical School. All patients suffered from degenerative disk disease of cervical or lumbar spine. Those patients with potential immune compromising medication (corticosteroids, cytostatic therapy) and i.v. application of gadolinium for magnetic resonance angiography were excluded. Heparinized (10 U/ml sodium-heparin, Liquemin N, Hoffmann-La Roche, Grenzach-Wyhlen, Germany) peripheral blood samples (5 ml) were drawn prior and immediately after MRI.

MRI was performed on a 1.5 T superconducting magnet (Sigma Horizon, General Electric Medical Systems, Milwaukee, WI, USA). A phased array coil was used enabling investigation of the spine without change of the patient position. The cervical spine was scanned in four cases, the lumbar spine in eight cases. Additional thoracic sections were performed on seven cases.

After an initial T1-weighted sagittal topogram, the sagittal T1- and T2-weighted sections were obtained using the following parameters.

Spin echo (SE)-weighted sections: time of repetition (TR) 500 ms, time of excitation (TE) 11 ms, slice thickness 3 mm, field of view 26 cm, matrix 256×256. For pre-saturation we used a 50 mm thick saturation band which was placed ventral to the spine.

Fast spin echo (FSE) T2-weighted sections: TR 2500 ms, TE 90 ms, echo gain 12, slice thickness 3 mm, field of view 26 cm, matrix

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Table 1

Percentage of neutrophils (mean (S.D.), range) producing superoxide anion (=rhodamine positive) during the RB following PMA, *E. coli*, FMLP or TNF- α /FMLP stimulation before and after MRI

	PMA	<i>E. coli</i>	FMLP	TNF- α /FMLP
Before MRI	46.8 (22.3)	48.3 (13.9)	12.0 (7.1)	31.7 (22.6)
Range	12.3–83.0	24.1–67.9	2–25.3	17.2–97.8
After MRI	56.2 (22.4)	50.5 (11.3)	12.3 (6.5)	26.5 (6.9)
Range	17.4–87.2	24.1–65.7	3.5–22.2	14.4–39.3
Difference	128.9 (25.7)	107.4 (19.5)	114.5 (35.8)	98.1 (29.2)
Range	103.4–178.4	88.2–153.7	63.0–175.0	40.2–139.2

The difference (% mean (S.D.), range) between the RB before and after MRI were calculated using Eq. 2.

256 \times 256. For pre-saturation, flow compensation was used to reduce artefacts of liquor pulsation in phase encoding direction.

In addition, transaxial T1- and T2-weighted sections were performed in the case of pathological findings. 15 sections were obtained with a slab thickness of 4 mm. The scanning time was between 10 and 25 min, depending on the number of sequences performed. With respect to the time for patient positioning and the time for planning, the following sequence patients were exposed to the MF for 25–40 minutes.

2.1. Sample preparations

We used the original protocol published by Rothe et al. [4]. Blood was layered on equal Ficoll quantity (Ficoll-Hypaque, density 1.077 g/dl, Biochrom, Berlin, Germany). The nucleated blood cells of the supernatant were harvested after 1 g sedimentation at 22°C for 45 min. Phosphate buffered saline (PBS, Dulbecco's without Ca²⁺ and MgCl₂, Gibco BRL, Eggenstein, Germany) was portioned (1 ml) in Eppendorf cups and heated to 37°C. 30 μ l of the leukocyte supernatant (containing an average of 5×10^5 cells/ml) were incubated with 1×10^{-3} mol DHR (MoBiTec, Goettingen, Germany) at 37°C for 5 min. The RB was induced with either 1×10^{-4} mol PMA (Sigma, Deisenhofen, Germany), 50 μ l *E. coli* (1×10^9 /ml, HB 101, Sigma), 1×10^{-7} mol FMLP (Sigma), or by priming with 1×10^{-6} mol recombinant TNF- α (Sigma) for 5 min, followed by stimulation with 1×10^{-7} mol FMLP. After 20 min the RB reaction was terminated by transferring the samples onto ice. Viability discrimination was performed by adding 3×10^{-3} mol propidium-iodide (PI, Serva, Heidelberg, Germany) just prior to the flow cytometry measurement. Negative controls without stimulation were applied to detect possible pre-activation of neutrophils.

2.2. Flow cytometry analyses

The samples were analyzed using a flow cytometer (FACScan, Becton Dickinson, Heidelberg, Germany). For each sample, 15 000 events were measured. The flow cytometer was equipped with an argon ion laser adjusted to a wave length of 488 nm. The rhodamine emission was filtered and measured within the spectrum of 515–545 nm by the corresponding photomultiplier (FL 1). The photomultiplier for FL 3 was used for measuring the PI emission in excess of 650 nm. Sideward scatter (SSC) and forward scatter (FSC) were assessed in linear mode: FL 1 and FL 3 in logarithmic mode without compensation. All of the results were obtained with the use of a constant photomultiplier gain value. Erythrocytes and cell debris were excluded according to a high threshold adjusted in the FSC signals. Data files were stored in list mode and analyzed in dot plots using PC-LYSYS software (Becton Dickinson). Neutrophils were included by setting a polygonal gate in FSC versus SSC. These gated cells were transferred to a FL 3/SSC dot plot for the exclusion of dead neutrophils, due to their high fluorescence in FL 3, resulting from the intracellular PI content. Finally, only vital neutrophils were included in a FL 1/SSC dot plot and the possible effect of MRI on the neutrophil RB was estimated at approximately 5000 vital neutrophils per sample. The fluorescence intensity was expressed by the mean channel of the rhodamine 123 fluorescence, which was calculated after setting a linear region on the peak signal in FL 1.

2.3. Statistical methods

The stimulation of the RB by PMA, *E. coli*, FMLP, or TNF- α /FMLP after MRI was compared to the respective stimulation before MRI. The values are expressed as the mean percentage and standard deviation (mean, SD) of superoxide anion producing neutrophils. De-

pending on the type of stimulation, the burst reaction has a known considerably high individual variation [5]. To compensate the individual variation, the percentage of RB inhibition by MRI was calculated using the formula:

$$\% \text{ difference} = \frac{\% \text{ rhodamine positive neutrophils after MRI}}{\% \text{ rhodamine positive neutrophils before MRI}} \times 100\% \quad (2)$$

The % difference which is the net effect of MRI was compared to 100%. Because numeric data showed a Gaussian distribution (tested with the Kolmogorov-Smirnov goodness of fit test), the student's *t*-test for paired samples was applied for the evaluation of differences with a 95% confidence limit (SPSS/PC V 6.3 software package, SPSS, Munich, Germany).

3. Results

The acquisition time of the MRI was 20–45 min (mean time 27.6 ± 11.4 min). Pre-stimulation of neutrophils could be excluded in the negative controls without RB stimulation ($< 5\%$ in all samples). The percentage of PI positive, e.g. necrotic neutrophils was below 1% for all samples. No significant differences could be found between the percentage of superoxide anion producing neutrophils before and after MRI, regardless whether stimulation was performed with PMA, *E. coli*, FMLP, or TNF- α /FMLP (Table 1). Also the % difference of individual variation of the RB and respective fluorescence intensities expressed as the mean channels showed no significant difference between before and after MRI.

4. Discussion

A study recently demonstrated a statistically significant increase in the superoxide anion production in the presence of a low frequency, low intensity MF during PMA-induced RB of primed rat peritoneal neutrophils [2]. The authors used a dual monochromator spectrophotofluorimeter to measure the extension of DCF, resulting from DCFH, after reaction with free radical-derived oxidants. They believe that their results represent the first observation of MF influencing free radical production in living cells. Nevertheless, this study differentiates too greatly to be compared with ours. However, this particular investigation encouraged us to study the potential effects of the static high intensity MF during MRI on the RB of neutrophils in patients undergoing MRI. Besides the non-physiologic stimulus with a phorbol ester (PMA), the RB of neutrophils was stimulated by bacterial phagocytosis (*E. coli*), receptor activation with a bacterial peptide (FMLP), or cytokine priming (TNF- α) followed by FMLP activation. Phorbol ester stimulation led to the direct activation of protein kinase C. Phagocytosis raises the intracellular Ca²⁺ content, leading to the activation of protein kinase C followed by NADPH

oxidase activation [5]. Priming with TNF- α was suggested to upregulate the neutrophil FMLP receptor expression [6]. Priming is defined as the exposure of neutrophils to a triggering agent so that there is a markedly increased response to a second stimulus. FMLP itself binds to a formyl-peptide receptor, leading to changes in the intracellular calcium concentration followed by oxidase activation [5]. Irrespective to the stimulating pathway, we were unable to prove an effect on the RB of human neutrophils after a mean time of 27.6 min of MRI.

To our knowledge, only one other study by Minczykowski et al. [7] has aimed to investigate the possible effects of MRI on neutrophil function. In contrast to our results, the authors found that neutrophil functions of patients who underwent MRI with a 1.0 T MR tomograph were seriously modified. A decrease was discovered in the peripheral neutrophil count together with an increased rate of phagocytosis. Compared with controls, they found no change in the superoxide anion production, apart from an increase in the H₂O₂ production from PMNs isolated from the blood of patients exposed to MRI and from blood samples that were left beside patients during MRI. Also, after stimulation with zymosan, the samples exposed to MRI produced significantly higher amounts of H₂O₂ than did the control samples. The production of stimulated and unstimulated H₂O₂ was applied with phenol red and horseradish peroxidase. In contrast, we were unable to prove these effects of MRI on neutrophil RB despite three different ways of stimulation: direct activation of protein kinase C by PMA, activation by phagocytosis of *E. coli* and receptor activation by FMLP after priming with TNF- α . Furthermore, in our study the phagocytic activity of neutrophils was indirectly tested with the stimulation of the RB by phagocytosis of *E. coli*. There was no significant difference between the *E. coli*-induced RB before and after MRI. This is also in contrast to the results of Minczykowski et al. who reported an increase in phagocytic activity after MRI.

An explanation for the described discrepancy in the potential effects of MRI on neutrophil functions between the data of the study of Minczykowski et al. and our results may be the differences in the applied techniques and the irritability of the RB enzyme complex.

In our study a multiparameter flow cytometry technique was used with DHR, which transforms into green fluorescent dye rhodamine in the presence of H₂O₂ as a result of the RB reaction. Additionally, neutrophil viability tested with the DNA dye PI was not influenced by MRI. PI can only adhere to the cellular DNA after destruction or disintegration of the cell membrane, which usually occurs subsequent to cell death. Therefore, only PI negative cells, hence viable cells, were in-

cluded in the estimation and cell toxicity could be excluded during the evaluation of oxygen radical production. A possible pre-activation of the RB induced by cell preparation or MRI could also be excluded.

The applied multiparameter flow cytometry technique has several advantages compared to other methodologies measuring the oxidative metabolism of neutrophils, such as chemiluminescence, horseradish peroxidase or cytochrome *c* reduction. With the present flow cytometry technique, it is possible to measure the intracellular activity of the RB oxidase enzyme complex. Four parameters of 15000 cells per sample were simultaneously assessed. Moreover, one specific cell population can be selected due to its specific scatter signals. Therefore, the RB was examined in approximately 5000 viable neutrophils. The objectivity of the procedure was warranted by the repeated adjustment of the equipment used and the use of external and internal positive and negative controls. Additionally, it has been shown that the DHR flow cytometric assay is the most sensitive technique available for investigating changes in oxidative activities of neutrophils compared to DCFH, chemiluminescence and spectrophotometric assays [8]. Furthermore, the cell separation technique can influence the neutrophil function. We used Ficoll sedimentation, which has the advantage that potential pre-activation occurring by centrifugation or lysis of erythrocytes can be excluded [9].

In conclusion, with a commonly accepted method, we could not prove an effect of short time exposure to a 1.5 T MF during MRI on the RB of neutrophils in a small population of 12 patients. We believe that further research is needed to assess the potential effects of MRI on neutrophil functions.

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