Decrease of respiratory burst in neutrophils of patients with ankylosing spondylitis by combined radon-hyperthermia treatment

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ABSTRACT
Objective
To define the respiratory burst activity of neutrophils, the total anti-oxidative status of plasma, and the parameters of systemic inflammation in patients with ankylosing spondylitis (AS) before and after a combined radon-hyperthermia treatment in the thermal tunnels of Böckstein-Bad Gastein in Austria.

Methods
In 20 patients with AS the effects of a total of 15 hours of radon-hyperthermia-treatment spread over a period of three weeks were studied. The respiratory burst activity of neutrophils was measured fluorometrically using dichlorofluorescein diacetate, the total anti-oxidant status was measured using azinodiethyl-benzthiazoline-sulphonate, and inflammation parameters were determined by routine laboratory assays.

Results
Before treatment, the basal neutrophil respiratory burst in patients (n = 20) was 409 ± 62 fluorescence arbitrary units (AU; mean ± SEM) and 359 ± 37 AU in controls (n = 9; p > 0.5); the stimulated respiratory burst (fMet-Leu-Phe, 10⁻⁶ M) was 1,027 ± 133 AU in patients and 1,152 ± 218 AU in controls (p > 0.5). After treatment, the basal neutrophil respiratory burst in patients (n = 19) was 137 ± 16 and in controls it was 174 ± 35 AU (n = 8; p > 0.1); the stimulated respiratory burst was 670 ± 66 and 1,305 ± 8 AU, in patients and controls respectively (p < 0.001). No effects of treatment on the total anti-oxidant status of the plasma or on the parameters of inflammation were detected.

Conclusion
Combined radon-hyperthermia treatment reduces the respiratory burst activity of the blood circulating neutrophils in patients with AS. If respiratory burst activity from the neutrophils plays a role in the pathophysiology of ankylosing spondylitis, the observed reduction may be related to the benefical effects of radon-hyperthermia treatment.
current medication was recorded, as was the clinical activity. At the time of the analyses, 12 of the 20 patients were receiving NSAIDs, while one was being treated with intravenous injections of corticosteroids (for four days during the period of combined radon-hyperthermia treatment; total dose 20 mg methylprednisolone), and one was using a corticosteroid aerosol because of chronic obstructive lung disease.

Combined radon-hyperthermia treatment was performed in the thermal tunnels of Bockstein-Bad Gastein for three weeks, with the administration of 10 treatments of 90 minutes each at 37.0°C to 41.5°C ambient temperature, 70% to 95% air humidity and about 4.5 nCi of radon per liter of air (6). The control group (4 women, 5 men) aged 44 ± 5.1 years (range 38 - 50) consisted of healthy volunteers with no medication and no exposure to radon and hyperthermia. Forearm venous blood samples were taken at the beginning and at the end of the treatment period. For different reasons at the end of the treatment period, one sample of blood from each patient and control became unavailable for analysis.

Neutrophil isolation
Neutrophils were obtained from the peripheral blood after discontinuous density gradient centrifugation on Percoll by dextran sedimentation and centrifugation through a layer of Ficoll-Hypaque, followed by hypotonic lysis of contaminating erythrocytes using sodium chloride solution (7). Cell preparations yielded > 95% neutrophils (by morphology in Giemsa stains) and > 99% viability (by trypan dye exclusion).

Respiratory burst of neutrophils
The respiratory burst activity of neutrophils was assayed with 2',7'-dichlorofluorescein diacetate (DCFH-DA). This assay is based on the oxidation of non-fluorescent DCFH-DA to highly fluorescent 2',7'-dichlorofluorescein both intracellularly and extracellularly (8). 100 µL/well (96-well plate, Falcon 3072) of 2 x 10^5 neutrophils were immersed at 37°C in a 1 x 10^{-5} mol/L solution of DCFH-DA in phenol red-free Hanks’ balanced salt solution containing 1 µmol/L of formyl-Met-Leu-Phe as a trigger-agent, or medium. The plates were covered with lids and placed in a humidified incubator (95% air/5% CO_2) for 20 minutes. Fluorescence activity was determined at 485 nm excitation and 530 nm emission wavelengths using the CytoFluor 2350 fluorescence measurement system (Millipore Corp., Bedford, MA).

Total anti-oxidant status of plasma and parameters of inflammation
The total anti-oxidant status (9) was measured photometrically at the 600 nm wavelength using azinoethylbenzthiazoline sulphonate and met-myoglobin according to the manufacturer’s instructions (WAK-Chemie Medical, Bad Homburg, Germany). ESR, CRP, IL-1, sIL-2r and neopterin were analyzed by standard laboratory techniques.

Statistics
Data are expressed as means ± SEM. Statistical analyses were performed using the unpaired 2-tailed Student’s t-test and multiple group comparisons using the Kruskal-Wallis-test (StatView software package; Abacus Concepts, Berkeley, CA).

Results
As shown in Figure 1, the basal neutrophil respiratory burst (formyl-Met-Leu-Phe, 10^{-6} M) was 1,027 ± 133 AU in patients and 1,152 ± 218 AU in controls (p > 0.5). After treatment, the basal respiratory burst in the neutrophils of patients (n = 19) was 137 ± 16 AU and in controls (n = 8) it was 174 ± 35 AU (p > 0.1), whereas the stimulated respiratory burst was 670 ± 66 and 1,305 ± 82 in the patients and controls, respectively (p < 0.001). No differences in the total anti-oxidant status were detected between the plasma samples of patients before (2.14 ± 0.05 mmol/L; n = 20) and after therapy (2.08 ± 0.036 mmol/L; n = 19), nor in the plas-

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**Fig. 1.** Basal and formyl-Met-Leu-Phe (fMLP)-stimulated respiratory burst activity of peripheral blood neutrophils from patients with ankylosing spondylitis before and after combined radon-hyperthermia treatment and from healthy controls. Respiratory burst activity was measured fluorometrically. Controls before treatment of patients, n = 9; controls after treatment of patients, n = 8. Patients before treatment, n = 20; patients after treatment, n = 19. ***p < 0.001 (two-tailed Student’s t-test for unpaired samples).
ma samples of the controls pre- and post-treatment (2.02 ± 0.026 mmol/L, 1.97 ± 0.024 mmol/L; n = 9; Kruskal-Wallis-test, p > 0.05).

All of the parameters of general inflammation including the ESR, CRP, IL-1, sIL-2r and neopterin were within normal ranges, and no differences were observed in relation to the control group either before or after treatment (Table I).

Discussion

Superoxide anions are believed to be implicated in the tissue damage in rheumatic diseases. The exact mechanism of these effects is not fully understood, but there is evidence for the involvement of inflammatory mediators and medication in these processes (10). In AS, data on oxidative bursts are not consistent, which might be explained firstly by the different test systems employed, and secondly by the use of various stimulatory agents at different concentrations in the various studies (1, 3, 4, 11). Furthermore, the reduced superoxide anion release from neutrophils described in some patients with AS may be attributed to the pathophysiology of the disease, but it could just as well represent the consequence of treatment with NSAIDs (3).

In our study, resting and stimulated superoxide anion release from the neutrophils of AS patients before combined radon-hyperthermia treatment in the thermal tunnels of Bockstein-Bad Gast ein were not different from stimulated superoxide anion release from the neutrophils of control subjects. This observation may be due to the frequency of NSAID therapy in the patient population, i.e. 12 out of 20 were on NSAID treatment (3). In addition, the total anti-oxidative status of the plasma was not different between patients and controls, either before or after the combined radon-hyperthermia treatment. In a previous study, the superoxide dismutase activity of neutrophils was found to be reduced in the neutrophils of patients with rheumatic diseases, including AS (12). Our findings in plasma suggest that in the systemic circulation of AS patients the total anti-oxidant status is not affected.

As a significant observation, we report an ex vivo reduction in superoxide anion release from the neutrophils in AS patients after combined radon-hyperthermia treatment. A total of 15 hours of exposure to radon-hyperthermia over a period of 21 days reduced the amount of stimulated superoxide anion release from the neutrophils of AS patients by about 30% as compared to pre-treatment values. This reduction was independent of the neutrophils of patients with AS. Our findings in plasma suggest that in the systemic circulation of AS patients the total anti-oxidant status is not affected.

The fact that no modification was made in the anti-inflammatory medication of the patients during the course of the treatment protocol suggests that exposure to radon-hyperthermia was responsible for the observed reduction in the respiratory burst.

Clinical benefits from combined radon-hyperthermia treatment have been documented previously (16), although the underlying mechanisms of these effects remain unknown. The observation of reduced neutrophil respiratory burst activity after combined radon-hyperthermia treatment provides a pathophysiological rationale for the treatment under the premise that the respiratory burst activity of blood circulating neutrophils is in fact related to the chronic inflammation in AS.

References


