

Qi-training Enhances Respiratory Burst Function and Adhesive Capacity of Neutrophils in Young Adults: A Preliminary Study

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Abstract: The main objective of this study is to examine the effect of Qi-training on the immune system, especially neutrophil bactericidal function. Nine healthy male subjects were studied for the effects of one bout of *ChunDoSunBup* (CDSB) Qi-training on superoxide (O_2^-) production and adhesion capacity of neutrophils at times immediately after (Post I) and 2 hours after the Qi-training (Post II). The Qi-training enhanced the O_2^- production, reaction velocity and neutrophil adhesion capacity and there were significant differences at Post I compared to before Qi-training (Pre). In addition, the number of white blood cells (WBC), monocytes and lymphocytes were changed significantly through Qi-training. Therefore, it seems that CDSB Qi-training may increase the resistance of trained individuals against common infection and inflammation.

Keywords: Qi-Training; Qigong; Neutrophils; Superoxide Generation; Adhesion.

Introduction

The effect of exercise on immunity is an important health issue that encompasses a wide range of activities from light exercise to intensive training programs (Brines *et al.*, 1996; Nieman, 1997; Nieman and Pedersen, 1999; Pedersen and Hoffman-Goetz, 2000; Woods

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et al., 1999). It is well known that moderate exercise may enhance resistance to infection and inflammation. On the other hand, it has been reported that intensive and strenuous exercise decreases resistance to infection. Neutrophils, which constitute 60–70% of the total circulating white blood cell (WBC) population, are the first line of defense against foreign microorganisms (Ortega Rincon, 1994). Neutrophils represent one of the various immune cells that modulate bactericidal and fungicidal function. Neutrophils contact phagocytes and microbes and ingest them with toxic reactive oxygen intermediates and hydrolytic enzymes. Recently, we have reported that *ChunDoSunBup* (CDSB) Qi-training produces regulatory functions in the neuroendocrine and immune systems (Lee *et al.*, 1999; Ryu *et al.*, 1995 and 2000a). CDSB Qi-training is composed of light intensity of motion (13% of VO_{2max}) and meditation (Kim *et al.*, 1996). But, the change of the innate immunity after acute Qi-training has not been examined. Therefore, the present study questions whether Qi training is sufficient to modulate the functions of neutrophils or other immune cells. We investigated the effect of Qi-training on the superoxide (O_2^-) production and adhesion capacity of neutrophils.

Materials and Methods

Subjects

The experiment was conducted on nine male volunteers engaged in CDSB Qi-training at Chunju branch, Korea. Their mean (\pm SD) physical characteristics were: age 26 ± 4 years, height 170 ± 4 cm, weight 60 ± 5 kg and they had trained 1.0 ± 0.5 years. The study protocol was approved by ethics committee of the Human Subjects Review Board at Wonkwang University Hospital and School of Medicine, and all the subjects gave their informed consent. They abstained from smoking and liquids with caffeine or alcohol for at least 6 hours prior to testing. All subjects were in good health without a history (remote or recent) of chronic disease, malnutrition, malignancy or renal disease. None was taking any medications such as steroid hormones that might affect physical activity.

Materials

Hank's balanced salt solution (HBSS) and phosphate-buffered saline (PBS) were prepared by conventional methods and filtered. HBSS contained 5 mM D-glucose. A single batch of opsonized zymosan (used for all experiments) was prepared by incubating 10 mg of Zymosan-A particles (highly glycosylated fragments of yeast cell walls) (Sigma, St. Louis, MO) with 1 ml of fresh human plasma at 37°C for 30 minutes. The suspension was centrifuged at 400 g for 2 minutes and the pellet washed twice with PBS before resuspension in PBS at a final concentration of 10 mg/ml. Aliquots (1 ml) were stored frozen and used only once after thawing. Counting by hemocytometer revealed that this preparation contained approximately 10^6 zymosan particles/ μl .

Polymorphonuclear Leukocytes Isolation

Human venous blood (10 ml) was collected from healthy adult volunteers into heparinized tubes. To obtain neutrophils from peripheral blood, the heparinized blood was centrifuged for 10 minutes at 1000 rpm to remove platelet-rich plasma. After 2% dextran sedimentation of erythrocytes for 30 minutes, neutrophils were isolated under sterile conditions by density gradient centrifugation on Ficoll-Paque cushions in conical tubes. The tubes were centrifuged at 2500 rpm for 30 minutes in swing-out buckets at room temperature. Contaminating erythrocytes were lysed with hypotonic solution containing NH_4Cl -EDTA and then washed twice. The cells were gently resuspended in magnesium-free HBSS containing 1.6 mM CaCl_2 ; then the cell number and viability were determined. The entire procedure was conducted in sterile conditions at room temperature. The final cell preparation comprised at least 97% neutrophils and < 0.2% monocytes, as assessed by Wright-Giemsa differential staining. The viability of neutrophils was > 98% as determined by trypan blue exclusion.

Measurement of Cell Adhesion

Neutrophil adhesion to 96-well tissue culture plates was evaluated as described by Nagata *et al.* (1995). Briefly, non-adherent cells were decanted immediately following the incubation with the reagent, RPMI-1640 medium (Sigma Co., St. Louis, MO), and the reaction wells were rigorously washed three times with 37°C HBSS. A total of 200 μl of 0.5% crystal violet in 12% neutral formaldehyde solution and 10% ethanol were then added to each well for 1 hour to fix and stain cells. The samples were thoroughly washed with water and air-dried for 30 minutes. Crystal violet was extracted by the addition of 1% SDS, and absorbency was measured at 570 nm. Adhesion was expressed as increased absorbency at 570 nm.

Measurement of Superoxide Anion (O_2^-) Production

O_2^- was measured by chemiluminescence assays. Ten microliters of lucigenin (10, 10'-dimethyl-9,9'-acridinum) (Sigma) (0.3 mM final concentration), as a chemiluminescence source, was added to each tube containing 1×10^6 cells in a 300 μl volume of Veronal buffer (containing BSA-Bovine serum albumin, glucose, Ca^{2+} and Mg^{2+}). The tubes were immediately placed in a lightproof and thermostatic chamber of a six-channel Biolumant LB9505 (Berthold, Bad Wildbad, Germany) and stabilized for 20 minutes. For the enhancement of O_2^- production, the triggering agent, opsonized zymosan (50 particles/cell) (Sigma Co., St. Louis, MO) was added to the tubes. The number of resultant photons from lucigenin-dependent chemiluminescence was recorded in counts per minute (cpm) for 60 minutes.

Reaction Velocity

In order to measure the kinetics of superoxide generation, we analyzed the peak chemiluminescence versus maximal response time.

Reaction velocity = peak chemiluminescence / maximal response time

Experimental Procedure and Blood Sampling

CDSB Qi-training which has previously been reported by Ryu *et al.* (1995) was performed by the subjects for 1 hour and was directed by a master instructor. One hour of Qi-training consisted of resting for 10 minutes before Qi-training and three kinds of exercise (sound (*Chunmoon*) reciting for 15 minutes, slow motions for 15 minutes, and meditation for 20 minutes). The peripheral blood was obtained by venipuncture using heparinized (10 U/ml) syringes from the median cubital vein. In order to determine the optimal sampling times, three trained individuals participated in *CDSB* Qi-training. From this preliminary data, three sampling times were selected for all subsequent experiments to minimize the number of blood drawings. Blood was drawn before Qi-training (Pre, that is 10 minutes before Qi-training), immediately after the Qi-training (Post I, within 10 minutes after Qi-training) and 2 hours after Qi-training (Post II). The samples were immediately treated for separating neutrophils by the above-mentioned methods.

Statistical Analysis

Data are presented as the mean \pm SD. One-way repeated ANOVA was used to evaluate the statistical differences between sampling times. If this analysis showed a significant effect ($p < 0.05$), subsequent comparison was performed using Scheffe's test.

Results

The responsiveness of neutrophils isolated from the peripheral blood of Qi-trained subjects to opsonized zymosan was changed by Qi-training [$F(2, 16) = 9.94$, $p = 0.005$] (Fig. 1). Superoxide generation was enhanced significantly immediately after Qi-training (Post I) compared to before Qi-training (Pre) ($p < 0.001$). The enhancement of superoxide generation induced by Qi-training (1.3-fold increase at Post I) intended to return to the baseline 2 hours later (Post II), although somewhat increased when compared to Pre ($p > 0.05$). There was a significant difference at Post II compared to Post I ($p < 0.05$).

Table 1 presented the mean and standard deviation of kinetics and neutrophil adhesion. One-way repeated ANOVA revealed significant effects of Qi-training on reaction velocity [$F(2, 16) = 4.95$, $p = 0.02$] and neutrophil adhesive capacity [$F(2, 16) = 3.92$, $p = 0.04$]. There was a significant change at Post I compared to Pre ($p < 0.05$) in reaction velocity. The reaction velocity returned to baseline 2 hours after Qi training. The adhesive capacity of neutrophils was enhanced significantly immediately after Qi-training ($p < 0.05$). Further, the values remained at the elevated level 2 hours after Qi-training.

The total number of WBC as well as the neutrophil, monocyte and lymphocyte concentrations in relation to Qi training are shown in Table 2. There were significant increases in the number of WBC [$F(2, 16) = 11.27$, $p < 0.001$], monocytes [$F(2, 16) = 14.31$, $p < 0.001$] and lymphocytes [$F(2, 16) = 4.6$, $p = 0.026$] after Qi-training. There were significant differences among the stages of the experiment (Pre versus Post II, $p < 0.01$; Post I versus Post, $p < 0.01$) in WBC number. Scheffe's comparison showed that the number of monocytes

was significantly changed at Post II compared to Pre ($p < 0.01$) and Post I ($p < 0.01$). The number of lymphocytes was increased at Post II compared to Post I ($p < 0.05$).

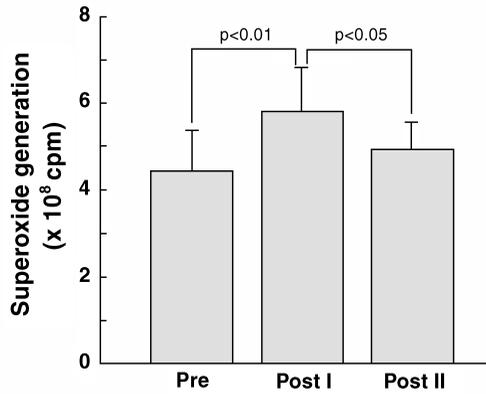


Figure 1. Effect of Qi-training on superoxide (O_2^-) generation by zymozan-stimulated neutrophils. Typical responses were measured before (Pre), immediately after (Post I) and 2 hours after Qi-training (Post II). Significance was analyzed by repeated measured ANOVA with Scheffe's test.

Table 1. Effects of Qi-training on Reaction Velocity and Adhesion of Neutrophils

Neutrophil Functions	Time			Analysis	
	Pre	Post I	Post II	F(2,16)	P
Reaction velocity (10^5 cpm/sec)	0.549 ± 0.194	0.732 ± 0.235*	0.606 ± 0.188	4.95	0.02
Neutrophils Adhesion	0.186 ± 0.047	0.227 ± 0.045*	0.212 ± 0.030	3.92	0.04

Values are expressed as mean ± SD (n = 9). Pre: Before Qi-training; Post I: Immediately after Qi-training; Post II: Two hours after Qi-training. *Significantly different from Pre at $p < 0.05$ by Scheffe's comparison test.

Table 2. Effects of Qi-training on Number of Immune Cells

Cell Type (10^3 cells/mm ³)	Time			Analysis	
	Pre	Post I	Post II	F(2,16)	P
WBC	6.082 ± 0.360	6.145 ± 0.557	6.541 ± 0.610*‡	11.27	< 0.001
Neutrophils	3.169 ± 0.582	3.127 ± 0.673	3.441 ± 0.679	2.36	0.123
Monocytes	0.692 ± 0.340	0.849 ± 0.405*	0.674 ± 0.378‡	14.31	< 0.001
Lymphocytes	2.189 ± 0.383	2.131 ± 0.203	2.393 ± 0.313‡	4.60	0.026

Values are expressed as mean ± SD (n = 9). Pre: Before Qi-training; Post I: Immediately after Qi-training; Post II: Two hours after Qi-training. * $p < 0.01$; †Significantly different from Pre; ‡ $p < 0.05$; ‡ $p < 0.01$: Significantly different from Post I by Scheffe's comparison test.

Discussion

There is now substantial epidemiological evidence that a single bout of Qi-training (acute effects) and prolonged training over several months (chronic effects) can produce positive and significant changes in the psychological, neuroendocrine and immune systems (Lee *et al.*, 1999; Ryu *et al.*, 1995). It is proposed that Qi-training produces good psychological health and a better immune system via secretion of many kinds of neurohormones. Our present results demonstrate that a single session of Qi-training enhanced neutrophil oxidative burst activity and adhesion capacity. In addition, Qi-training increased the number of immune cells, including WBC, monocytes and lymphocytes.

The current findings show that Qi-training, a light intensity of Korean exercise, is sufficient to elevate the ability of circulating neutrophils to produce microbicidal reactive oxygen intermediate (superoxide anion, O_2^-) when stimulated with zymosan particles. Furthermore, this respiratory function is activated immediately after Qi-training faster than before Qi-training. Ryu *et al.* (1997) reported *in vitro* results that growth hormone (GH) can act through the increase of intercellular Ca^{2+} as priming agent to activate neutrophils for an enhanced respiratory burst. Other studies show that Qi-training acutely increases GH, IGF-I and IGFBP-3 in young adults, and the changes in neutrophil superoxide (O_2^-) production in response to Qi-training were mediated by the increased endogenous GH concentrations in aging man (Lee *et al.*, 1999; Ryu *et al.*, 1996 and 2000a). Thus, we proposed that enhanced superoxide generation and reaction velocity after Qi-training may be related to neurohormonal modulation that occurs through Qi-training.

The neutrophil response to infection includes adhesion, chemotaxis, phagocytosis, oxidative burst, degranulation and microbial killing (Ortega Rincon, 1994; Pyne, 1994). Neutrophil adhesion is fundamentally important during the onset of inflammatory responses. The adhesion signaling pathways control neutrophil arrest and extravasations and influence neutrophil shape and function at sites of inflammation. Hence, enhanced adhesive capacity by Qi-training may be proposed to increase immunosurveillance function.

Our group recently reported that pituitary GH modulates neutrophil adhesion through tyrosine phosphorylation of Jak2, p125^{FAK}, and paxillin and actin polymerization (Ryu *et al.*, 2000b). And it was reported that levels of GH were increased significantly during and after Qi-training (Lee *et al.*, 1999; Ryu *et al.*, 1996 and 2000a). Thus it is supposed that enhanced adhesion capacity following Qi-training may be related to the increase of GH level. Qi-training also coupled the nervous system to the endocrine system and possibly to the immune system since there was an increase of WBC, monocyte and lymphocyte numbers. These results are consistent with our recent finding that Qi-training significantly changes the numbers of lymphocyte and monocyte when compared with a placebo control (Lee *et al.*, in press).

In summary, our current observations show that Qi-training contributes to enhanced respiratory function, reaction velocity and adhesiveness of neutrophils. According to the neutrophil's function as a first line defense against foreign microorganisms, it is supposed that Qi-training may increase the resistance of trained individuals against common infections

and inflammation. Further research is required to determine which aspects of Qi-training contributed to these changes in neutrophil functions, and to ascertain whether the same amount of exercise in general would result in similar alterations, or if they were augmented by the meditative aspects of Qi-training. In addition, it is also necessary to study how age and duration of exercise might affect the magnitude of the neutrophil response, as well as clarify the mechanism of action.

Acknowledgments

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