Int.J. Behav. Med. (2016) 23:198–203
DOI 10.1007/s12529-015-9509-8

Acute Effects on the Counts of Innate and Adaptive Immune Response Cells After 1 Month of Taoist Qigong Practice

Francisca M. Vera[1] · Juan M. Manzaneque[1] · Francisco M. Rodríguez[2] · Rebecca Bendayan[1] · Nieves Fernández[3] · Antonio Alonso[3]

Published online: 14 September 2015

Abstract
Background Qigong is an ancient form of health maintenance, dating back thousands of years, which is part of Traditional Chinese Medicine. Numerous physical as well as mental benefits have been classically ascribed to this traditional mind-body method which integrates slow body movements, breathing, and meditation. Albeit we have already reported an immunomodulatory action of qigong in other investigations, measures were then assessed 1 day after the qigong program ended.
Purpose The aim of the present study was to assess the acute effects of Taoist qigong practice on immune cell counts in healthy subjects 1 h after training.
Method Forty-three healthy subjects participated in the study of whom 25 were randomly allocated to the experimental group and 18 to the control group. The experimental subjects underwent daily qigong training for 1 month. Blood samples for the quantification of immune parameters (number and percentage of monocytes, neutrophils, eosinophils, total lymphocytes, B lymphocytes, and natural killer (NK) cells) were taken the day before the experiment commenced and 1 h after the last session of the training program ended. As statistical analysis, analysis of covariance (ANCOVA) was performed.
Results Statistically significant differences were found between the experimental and control groups, with the experimental group showing higher values in the number (p=0.006) and the percentage (p=0.04) of B lymphocytes, as well as lower values in the percentage of NK cells (p=0.05), as compared to control.
Conclusion This study demonstrates that Taoist qigong is able to exert acute immunomodulatory effects on components of both innate as well as adaptive immune response.

Keywords Qigong · Psychosomatic · Holistic · Immune · Mind-body

✉ Francisca M. Vera
pvera@uma.es

[1] Universidad de Málaga, Andalucía Tech, Departamento de Psicobiología y Metodología de las Ciencias del Comportamiento, Campus de Teatinos s/n, 29071 Málaga, Spain

[2] Unidad de Gestión Clínica de Laboratorio AGS Este de Málaga-Axarquía, Málaga, Spain

[3] Servicio de Inmunología, Hospital Regional Universitario de Málaga, Avenida de Carlos Haya s/n, Málaga, Spain

Introduction

Qigong is an ancient psychosomatic discipline which is part of Traditional Chinese Medicine. It can be traced back thousands of years, being a highly popular practice nowadays, particularly in China, for the purpose of health maintenance and healing [1]. Qigong, of which there are hundreds of variations, aims at mind-body harmonization by integrating soft body movements, breathing, and meditation into a single multifaceted practice [2, 3]. According to the philosophy of Traditional Chinese Medicine, qigong training is designed to enhance qi function [4, 5] and regulate the functional activities of the body through regulated breathing, mindful concentration, and gentle movements [5]. Thus, practicing qigong simultaneously trains the mind, body, and qi (vital energy) for the benefits of physical, psychological, and spiritual health [6]. One of the main traditions within qigong is the Taoist school of qigong. This particular school emphasizes naturalness and uses specific body movements and a relaxed state of mind to improve health and increase longevity [7].

Qigong, commonly recognized for its holistic approach, has received considerable attention in biomedical research due to its remarkable psychobiological effects and its broad therapeutic potential. Related literature has reported many health-related benefits from the practice of qigong [8–16], including immunomodulatory effects [13, 14, 17–19].

Immune responses can be modulated by behavioral interventions via changes in the autonomic nervous system and neurohormonal secretion. Qigong can affect the psychological state, and this, in turn, influences the neuroendocrine system, whereby effects on immune cells can take place [17]. This psychosomatic training can improve immune function by stimulating homeostasis of the sympathetic and parasympathetic nervous systems through hypothalamic action [13]. Thus, the psychobiological mechanism of qigong may involve downregulation of the activity in the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis and modulation of the immune system, either by changing the number of immune cell types or by improving the functionality of the specific immune cells [20]. These changes of immune parameters have been also reported after yoga and meditation practice [21–23].

Despite scientific evidences on the immunomodulatory action of qigong, research on its acute immunological effects is limited. We have already published works on the effects of qigong on immune response, although biological measures were, then, assessed 1 day after the training program ended [17, 18].

Acute immunological effects after a short 30-min exercise session would imply an immediate modulation of the immune system. This immunomodulation, resulting from the quick activation of psychobiological mechanisms, would highlight the power of this psychosomatic method to induce significant physiological changes. Recently, Qu et al. [24] observed rapid (within 2 h of starting training) and significant gene expression changes in peripheral blood mononuclear cells of practitioners during a comprehensive yoga program. These data suggest, according to the authors, that these psychosomatic disciplines have an integral physiological component at the molecular level which is initiated immediately during practice and may form the basis for long-term stable effects. In this sense, we think that the aforementioned acute effects, by themselves, could be of considerable interest, particularly in a clinical context, given the nature and relevance of the physiological system displaying the changes, as well as the quickness for these to appear. In addition, although it has not been confirmed yet if acute changes are relevant to long-term health, it is indeed necessary to count on this type of information to be able to view a broader picture of the scope of effects that qigong can induce.

Given the modulatory action of qigong practice on the immune system, exploring its effects on immune parameters provides relevant information about its possible implications in the clinical field and health. Albeit this mind-body exercise has been demonstrated to exert a significant immunomodulatory action, there is a marked lack of scientific research in Western world about the effects of Taoist qigong. Moreover, qigong has been scarcely investigated with regard to its immediate effects on the immune system. Therefore, this research was consequently carried out for the purpose of shedding light on the immediate modulation of Taoist qigong on immune parameters, after 1 month of practice. The present study was specifically designed as an exploratory assessment of the acute effects of this psychosomatic method, 1 h after the last training session, on immune cell counts in healthy subjects.

Material and Methods

Participants

Forty-three subjects, aged 18–21 years, of whom 9 were male and 34 female, all psychology students at the University of Malaga, were selected from among a larger group of volunteers. Twenty-five subjects (20 female and 5 male) were randomly allocated to the experimental group and 18 (14 female and 4 male) to the control group. The percentage of male subjects in the experimental group was 20 %, while in the control group this percentage was 23 %; likewise, the percentage of female subjects in the control group was 80 %, while in the experimental group it was 77 %. Thus, the gender split was not greater than 3 %.

Only healthy subjects not taking any type of drugs and with regular life habits were chosen to be part of the experimental or control groups. None of them practiced sports regularly or had any previous experience with qigong, yoga, or other such methods. All were asked to follow their normal lifestyles while the experiment was being conducted. Subjects were screened to exclude those with any pathological conditions and/or those who had received pharmacological treatment in the 3 months prior to the experiment. All subjects were compensated for their participation on the study and received either 100 euros (experimental group) or 50 (control group). The different incentives to participants depending on the study condition were established owing to the fact that the degree of compromise was different for each condition. Thus, participants on the experimental group had to follow a more demanding program than the control group.

Female subjects were all within the first 10 days of their menstrual cycle. One experimental subject (female) decided to abandon the experiment within the first few days of onset, and a further four subjects (one male and three female) were lost the last day.

Informed consent was obtained from all participants in the study after the nature of the procedure had been fully explained to them.

Intervention

The form of qigong taught belongs to the Taoist school of qigong. It is a simple classical method containing several movements which are performed on a standard stance with feet flat on the ground, parallel, shoulder width apart. The whole physical sequence contains seven discrete movements which entails the stretching of the hands, arms, trunk, waist, and legs. Each movement is repeated six times, and no change of the basic feet position is to take place while performing the sequence. Throughout practice, natural, relaxed, and rhythmic breathing is required. This method of qigong reportedly dates back hundreds of years, although it has not yet been researched.

Experimental subjects underwent a qigong training program consisting of 3 days of practice per week plus some additional individual sessions, for the period of 1 month. These subjects gathered in the afternoon, on Monday, Wednesday, and Friday, in a room adjoining our laboratory where the practice sessions were conducted. The mind-body exercise took place and was taught under the guidance of a qualified qigong instructor of this discipline. Participants were asked to follow suit the slow movements shown by the instructor, with no other specific instruction given to them, except when questions by participants were placed. Each guided session lasted for 25–30 min and involved the performance of the aforementioned qigong sequence for two consecutive times with a 5-min rest in between. Subjects were encouraged, but not required, to keep practicing on their own at home. Thus, the training included the group sessions conducted by the instructor and some additional sessions carried out individually on the other days, including the weekends. The amount of individual training varied from one subject to another, but typically consisted of one extra session during the week plus one more on the weekend. This individual training usually had half the duration of the regular ones and consisted of one simple practice of the Taoist qigong sequence. In total, most subjects engaged in 20 qigong sessions, with a minimum of 15 sessions. During the experiment, control subjects were not required to do anything in particular and were just asked to follow their daily life habits.

Blood Sampling

The day before the experiment commenced and 1 h after the last session of practice concluded, 1 month later, blood samples were drawn from all subjects for the quantification of immune parameters. Blood was drawn by venipuncture at 0900–1000 hours.

The immunological parameters investigated included the number and percentage of monocytes, neutrophils, eosinophils, total lymphocytes, B lymphocytes, and natural killer (NK) cells.

The procedure was as follows:

- For total blood count (monocytes, neutrophils, eosinophils, and total lymphocytes), 5 ml of blood was introduced into an EDTA tube and analyzed by flow cytometric analysis in an ADVIA 2010 (Siemens) analyzer.
- For the immunophenotypification, all samples were evaluated by flow cytometric analysis with FACScan (Becton Dickinson) with a sorting module which allows the separation of the different lymphocyte subpopulations (B lymphocytes and NK cells).

Statistical Analyses

A between-group analysis of covariance (ANCOVA) was performed on several dependent variables: the numbers of monocytes, neutrophils, eosinophils, total lymphocytes, B lymphocytes, and NK cells as well as the percentages of monocytes, neutrophils, eosinophils, total lymphocytes, B lymphocytes, and NK cells. In general, all the immunological parameters were normally distributed except for monocytes and eosinophils, which presented a slight deviation from normality. However, ANCOVA has been found robust to violations of normality when homoscedasticity assumption is met [25]; in our data, this assumption was always met.

The qigong training was considered as an independent variable with two levels (absence or control group and presence or experimental group) and the respective pretest scores of each dependent variable as covariates. Thus, the differences between groups were estimated with the differences in pretest scores removed. A value of $p<0.05$ was considered to be significant. The alpha level was adjusted for multiple comparisons using the Bonferroni procedure. The software package IBM SPSS Statistics Version 19 was used for all analyses.

Results

One hour after completing the qigong program, the effects of experimental treatment, after adjustment for covariants, were found in the number and the percentage of NK cells and B lymphocytes. The adjusted means, F statistics, and p values are presented in Table 1. Specifically, it was found that the experimental group had lower values in the percentage of NK cells ($p=0.05$), as well as higher values in the number ($p=0.006$) and the percentage ($p=0.04$) of B lymphocytes, as compared to control.

Table 1 Adjusted means in posttest for the concentrations of immunological variables in the control and qigong groups after performing ANCOVA with respective pretest as covariants

Immunological parameters	Qigong (N=25)	Control (N=18)	F statistic	d.f.	p value
Neutrophils ($\times 10^3$ cells/μl)	3.97	4.13	0.25	1, 39	0.61
Monocytes ($\times 10^3$ cells/μl)	0.40	0.39	0.04	1, 39	0.83
Eosinophils ($\times 10^3$ cells/μl)	0.21	0.17	2.33	1, 39	0.13
Total lymphocytes ($\times 10^3$ cells/μl)	2.01	2.24	3.35	1, 39	0.07
B lymphocytes (cells/μl)	234.62	195.51	8.77	1, 39	0.006*
Natural killer cells (cells/μl)	337.70	388.79	2.41	1, 39	0.13
Neutrophils (%)	57.89	56.57	0.34	1, 39	0.56
Monocytes (%)	6.27	5.68	2.08	1, 39	0.15
Eosinophils (%)	3.31	2.80	1.71	1, 39	0.19
Total lymphocytes (%)	29.81	32.40	1.94	1, 39	0.17
B lymphocytes (%)	10.72	9.40	4.38	1, 39	0.04+
Natural killer cells (%)	16.26	18.96	3.83	1, 39	0.05#

*p=0.006

+p=0.04

#p=0.05

Discussion

The present investigation is the first one to demonstrate that Taoist qigong modulates immune cells in human peripheral blood. Our data showed a significant decrease in NK cells percentage and a marked increase in the number and percentage of B lymphocytes, as compared to the control group. These results, therefore, reveal that 1 month of Taoist qigong practice exerts a significant acute immunomodulatory action on two particular and relevant cell components of innate and adaptive immune response.

Researches about the acute effects of qigong on NK cell numbers are very limited. We have found only one study showing a significant reduction in the count of these cells [26]. This result can be considered similar to that found in our study, since a significant reduction in the proportion of NK cells was observed, although the number of NK cells did not change. Interestingly, unlike these results, Lee et al. [27] did not report significant changes in the count of this immune parameter after qigong practice, although an increase of cytotoxic activity of NK cells was found. In our study, we cannot know whether or not the change observed in the proportion of NK cells was accompanied by changes of its cytotoxic activity, since this was not assessed. However, it would be interesting to take this into consideration for future research.

While ours is the first study to evaluate the effects of Taoist qigong practice in NK cells, in our previous study, employing a different qigong method, we reported, similarly, a significant decrease on innate immune response cells 1 day after the program ended [17]. NK cells are key components of the innate immune system. Decreased or absent NK cell numbers or activity is often associated with the development or progression of cancer, acute or chronic viral infections, autoimmune diseases, immunodeficiency syndromes, and psychiatric illness [28]. On the other hand, physical as well as psychological stress increase the number of circulating peripheral blood NK cells [29, 30].

It has been reported that exercise-induced catecholamines may be involved in changes of circulating NK cells that express beta-adrenergic receptors [31]. Indeed, this important component is the most responsive immune cell to acute exercise [32]. According to research, the direction of these changes can differ. Specifically, Millard et al. [30] found that a brief exercise increased peripheral blood NK cell counts without immediate functional changes. On the contrary, Nagao et al. [31] described a reduction of NK cell number, 30–60 min after doing a 30-min workout on a cycle ergometer. Timmons and Cieslak [32] carried out a review on studies that measured NK subsets in peripheral blood collected just before and immediately after an acute bout of exercise. These authors argue that the time of a blood sample during exercise and the intensity at which the exercise is performed are important when interpreting the NK cell response. The mobilization of NK cells on blood circulation is relatively rapid and complete after 30 min of exercise. Taking this data into account, and given that Taoist qigong training frequently entails a series of slow physical movements, this psychosomatic exercise could account for the change on the NK cell percentage observed in the experimental group 1 h after training. Interestingly, this change returns to normal after 24 h (data not published in this article).

In our study, a decrease in NK cell percentage occurred, while, at the same time, a significant increment in the percentage and count of B lymphocytes was found. According to literature, NK cells are more sensitive to changes after exercise whereas the percentage of B lymphocytes does not seem to be affected in a similar way by physical activity [33]. In this context, it has been suggested that B lymphocytes could be one type of immune parameter more sensitive to relaxation

interventions than other kinds of immune parameters [26]. Thus, the relaxed state of mind induced by qigong may be an important element of this single multifaceted practice, which could explain the modulatory action of qigong in this immune component.

As far as we know, the present study is the first one reporting a significant change in B lymphocytes. Thus, while an increase in the number of lymphocytes after qigong practice has been previously described [26, 34, 35], there is no available evidence about the possible influence of qigong on this particular adaptive immune response cell. B lymphocytes are central components of adaptive immunity, responding to pathogens by proliferation, differentiation, and production of antibodies [36]. These immune cells are effectors of humoral immunity, providing defense against pathogens through different functions including antibody production [37]. In our previous study, using another method of qigong, we found a significant decrease in the total number of leucocytes 1 day after the program ended [17], particularly in the number of monocytes and granulocytes, whereas the number of adaptive immune response cells remained unaffected. Therefore, this is our first study reporting an immunomodulation of qigong practice on the adaptive immunity cells.

The reason for the different responses on individual immune cells found in our study is not known. It is likely that the immune components evaluated are sensitive, to a greater or lesser extent, to different elements integrated into qigong training. The marked complexity of the immune system, with its large variety of components, makes it difficult to point to a single and simple possible explanation for the results found. Thus, more knowledge for interpreting the immunomodulatory action of qigong in terms of beneficial health effects is needed.

It is also necessary to acknowledge that the small number of subjects employed in this study, the use of just two measures for the blood tests, as well as the time points for these blood measures offer, to a certain extent, some limitation to the overall results. It would be necessary that future studies should include a larger sample size, additional blood measures, as well as different time points for them. This would certainly provide a broader perspective and a better understanding of the nature of the effects induced by Taoist qigong on immune parameters. Further studies should also examine the effects of demographic factors on parameters of immune function as they were not explored in the present study.

In conclusion, our results reveal that Taoist qigong can affect the peripheral circulation of immune blood cells, exerting acute immunomodulatory effects on cell components of adaptive and innate immune responses. Further studies should confirm these results and fully assess its potential implications for human health care. Therefore, we consider interesting to continue exploring the promising utility of qigong as a complementary mind-body intervention.

Compliance with Ethical Standards

Consent to Participate Informed consent was obtained from all participants in the study after the nature of the procedure had been fully explained to them.

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