

## A Preliminary Study of the Effect of External *Qigong* on Lymphoma Growth in Mice

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### ABSTRACT

**Objective:** To examine the effectiveness of external *qigong* on the *in vivo* growth of transplantable murine lymphoma cells in mice.

**Background:** *Qigong* is a traditional Chinese health practice that is believed by many to have special preventive and healing power. Underlying the system is the belief in the existence of a subtle energy (*qi*), which circulates throughout the body, and when strengthened or balanced, can improve health and ward off or slow the progress of disease. To date, much of the literature showing the effects of *qi* are presented in the non-Western literature, and as such are viewed with considerable skepticism. In an attempt to demonstrate *qi* in a controlled setting, the effect of external *qigong* emission from a *qigong* healer on the *in vivo* growth of transplantable murine lymphoma cells in mice was explored in two pilot studies.

**Methods:** In study 1, 30 SJL/J mice were injected intravenously with lymphoma cells that localize and exhibit aggressive growth in the lymphoid tissues of untreated syngeneic recipients. These tumor-injected mice were divided into 3 groups: (1) *qigong* treatment (administered by a *qigong* healer); (2) sham treatment; and (3) no-treatment control. The sham group received the same number of treatments from a person without training in *qigong*, who imitated the motions of the *qigong* healer. The control group received no treatment at all. In study 1, the mice were sacrificed on the 9th or 11th days after tumor-cell injection, and in study 2, the mice were sacrificed on the 10th and 13th days. Tumor growth in lymph nodes (LN) was estimated by LN weight expressed as a percentage of total body weight.

**Results:** In study 1, LNs from mice in the *qigong*-treated group were significantly smaller than LN from mice in either the control group or in the sham treatment group ( $p < 0.05$ ), suggesting that there was less tumor growth in the *qigong*-treated mice. In study 2, using the same design as study 1, the same pattern of difference found in study 1 emerged: LN ratio from mice in the *qigong*-treated group was smaller than that in either the control group or in the sham group. However, these results did not reach statistical significance, partially as a result of larger variances in all groups in this study.

**Conclusions:** These preliminary results, while still inconclusive, suggest that *qigong* treatment from one particular *qigong* practitioner might influence the growth of lymphoma cells negatively. Further studies with different practitioners, more repeated trials, and/or different tumor models are needed to further investigate the effects of external *qigong* on tumor growth in mice.

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An earlier version of the paper was presented at the First International Conference on Asian Therapies for Cancer, February 28–March 3, 2001, New York City.

## INTRODUCTION

**Q**IGONG (pronounced "chi kung") is a traditional Chinese health practice and healing technique that postulates the existence of a subtle energy (*qi*), which circulates throughout the body, and when strengthened or balanced, it can improve health and ward off or slow the progress of disease. *Qigong* is believed to aid in healing and maintaining health, and has been used for several thousand years. Today, many people around the world practice *qigong* for health maintenance. In addition, there are many reports in the Chinese literature on the use of *qigong* for the treatment of various diseases ranging from hypertension to cancer (reviewed by Sancier, 1996). Rigorous investigations of these claims are needed before Western medicine will adopt *qigong* as a useful medical treatment or health practice.

The word *qigong* is a combination of two ideas: "*qi*," which means breath of life or vital energy, and "*gong*," which means the skill of working with, or achievement. *Qigong* consists primarily of meditation, relaxation, guided imagery, biofeedback energy building, and mind-body integration through regulating body gesture, mind and breath. Practitioners are said to develop an awareness of *qi* sensations in their bodies and use their minds and intentions to guide the *qi* for the purposes of achieving health and self-healing. It is reported that skillful *qigong* practitioners can direct or emit their *qi* energy (external *qi*) for the purpose of healing others. The studies presented in this paper attempted to demonstrate the effects of *qigong* in an experimental cancer model in mice.

According to Traditional Chinese Medicine, good health is a result of a free-flowing, well-balanced energy system, while sickness or the experience of pain is the result of *qi* blockage or unbalanced energy in the body. Because there is no technology at present that can measure *qi* accurately, one feasible way to conduct research on *qigong* is to study the subjects or objects that are purportedly exposed to *qigong* energy and investigate any changes in or differences between a *qigong*-treated group and a control group. Although the physical nature of *qi* remains as yet unproven, there are some in-

triguing reports that suggest an association between possible physical, biophysical, and/or biochemical alterations, and "*qi* emission." For example, it is reported that *qigong* emission may enable the growth of Fab protein crystals (Yan et al., 1999), inhibit tumor growth in mice (Chen et al., 1997), accelerate seed germination (Bai et al., 2000), and change the conformation of biomolecules such as polyglutamic acid, polylysine, and metallothioneine (Chu et al., 1998). Unfortunately, some of these studies suffer from a lack of controls and appropriate blinding and have not been well replicated in Western countries. There is, however, a small but growing body of scientific evidence in the literature that suggests the physical existence of *qi*, as well as the healing power of *qigong* therapy (Agishi, 1998; Hisamitsu, et al., 1996; Iwao et al., 1999; Loh, 1999; Sancier, 1994, 1999; Sancier and Chow, 1989; Wirth et al., 1997; Wu et al., 1999).

It has been reported in the Chinese literature that the Chinese Taiji Five-Element *qigong*, a form of medical *qigong* developed by Mr. Binhui He has shown some therapeutic effects on experimental tumor models (Chen et al., 1997) as well as on human cancer (Zhang, 1997). In a collaborative study with scientists at Zhongshan University of Medicine in Guangzhou, China to test the effect of external *qigong* on laboratory mice injected with liver cancer cells, Mr. He emitted external *qi* over 10 mice for 10 minutes on 4 separate occasions. The study reported an average of more than 70% reduction in cancer growth (in size and weight) in the *qigong*-treated group, compared to the sham treatment group and the control group. The same experiment was repeated 3 times, with the average inhibitory rate in size and weight of tumor mass of 70.3%, 79.7%, and 78.7%, respectively ( $p < 0.001$ ) (Chen et al., 1997).

We wanted to verify these observations using a different animal model and to explore the effectiveness and mechanisms of this new therapeutic method in cancer treatment. Therefore, we invited Mr. Binhui He to the United States to collaborate in a series of studies to investigate the effects of his external *qi* emission on the *in vivo* growth of transplantable murine lymphoma cells in SJL/J mice. The results from two separate studies follow.

## MATERIALS AND METHODS

The mice used for these experiments were female SJL/J mice obtained from Jackson Laboratories (Bar Harbor, ME). They were 8–12 weeks' old, and weighed between 18 and 22 g. The animals were housed in the Association for the Assessment and Accreditation of Laboratory Animal Care-accredited Research Animal Facility at the University of Medicine and Dentistry of New Jersey and studies were approved by the Institutional Animal Care and Use Committee.

A transplantable RCS lymphoma cell line was used in this study. This cell line was originally derived from a primary tumor of SJL mice (Carswell et al., 1970) and is maintained by weekly intravenous passage of  $5\text{--}10 \times 10^6$  tumorous lymph node cells into 7–10-week-old syngeneic recipients.

Our laboratory (N.M.P.) has been studying this B-cell lymphoma model for many years, and we have published extensively on the nature of tumor cells and on the host immune response that these tumor cells induce in syngeneic recipients (Ponzio et al., 1997; Ponzio and Thorbecke, 2000; Tsiagbe et al. 1998). The lymphoma cells used for the experiments were obtained from the enlarged lymph nodes (LN) of these tumor-injected SJL donors. Single-cell suspensions were prepared, and the cells were washed by repeated centrifugation and resuspension in tissue culture medium. After the final wash, cells were counted and adjusted to a concentration such that the desired number of cells for intravenous injection was contained in 200  $\mu\text{L}$  of phosphate-buffered saline (PBS).

In both studies, mice were randomly assigned to one of three groups: (1) A *qigong* treatment group; (2) a sham treatment group; and (3) a no-treatment group. In the initial study, mice were injected with lymphoma cells 24 hours after their first treatment. In the second study, mice were injected with lymphoma cells 4 hours after their first treatment. The frequency of the subsequent treatments also differed in the two studies, and is explained in more detail in the Results section.

In both studies, mice were injected intravenously in the retro-orbital sinus with  $2 \times 10^4$  lymphoma cells, and were monitored for en-

largement of peripheral LNs and spleen. When tumor growth resulted in palpable enlargement of lymphoid organs in any group, 10 mice from each group were sacrificed to obtain quantitative data on tumor growth. These assay methods, developed in our laboratory, provide a consistently reliable estimate of tumor growth in recipient mice (Alisauskas and Ponzio, 1989). Tumor burden was estimated by weighing the spleen and selected peripheral LN (two cervical, two axillary, two brachial, two inguinal, first segment of the mesenteric), and expressing these weights as a percentage of body weight for each mouse.

A randomized dual-blind design (Caspi et al., 2000) was used in these experiments, wherein the mice were randomly assigned to one of three groups. The laboratory director and research assistant were not aware of which conditions the mice had been in, the *qigong* healer was not involved in organ collection or assays (but not blinded to the treatment), and the statistician was blinded to the experimental conditions (randomly selected code letters were used as labels for each condition in the analyses).

The external *qigong* treatment involved the *qigong* healer purportedly emitting external *qi* from the palms of both hands toward the mouse cage at a distance of 10–15 cm for 10 minutes in each session. During these sessions, the *qigong* healer occasionally used his eyes or simply his intention, instead of his palms, to direct his energy. The number of treatment sessions and schedule varied in each of the two studies, as described below.

Data were analyzed with analysis of variance to examine the overall difference. The Student-Newman-Keuls (SNK) procedure and Duncan's multiple comparison procedure were applied in *post hoc* tests to detect group differences.

## RESULTS

### Study 1

*Methods.* This experiment was carried out in December 1999 when the *qigong* healer (B.H.) visited the United States for a lecture tour. A total of 90 mice were randomly assigned to one of three groups (30 each) as described above. All mice were injected with lymphoma cells 1

day after the first *qigong* session. The *qigong* healer then supposedly emitted external *qi* toward the mouse cage for 10 minutes, every other day, for a total of five sessions. The sham treatment was given by an individual without training in *qigong*, who simply imitated the movements of the *qigong* healer. The mice were housed 5 per cage during all treatments. The *qigong* healer treated 2 cages (10 mice) at a time (except the preinjection treatment in which all 6 cages were treated at once). In this study, approximately one-third of the mice from each group were sacrificed on day 9 after three postinjection treatments (a total of 4 treatments including the preinjection treatment), another one-third of the mice from each group were sacrificed on day 11 after 4 postinjection treatments (a total of 5 treatments including the preinjection treatment), and the remaining mice were sacrificed at a later point in time to allow for observation of survival differences for each group.

**Results.** Table 1 presents the results of day 9 and day 11 assays in which organ weight is expressed as a percentage of body weight as a measure of tumor growth for all three groups. Among the mice sacrificed on day 9, tumor growth in LN was significantly less in the *qigong*-treated group than in either the control group or the sham-treated group ( $p < 0.05$ ). Group differences for tumor growth in the

spleen were not statistically significant. Among the mice sacrificed at day 11, tumor growth in both the LN and spleen in the *qigong*-treated group was significantly less than in the control group ( $p < 0.05$ ). However, the difference between the *qigong*-treated group and sham-treated group did not achieve statistical significance.

The remaining one-third of the mice in this series were sacrificed on day 36 after tumor cell injection. By this time, mice in all three of the groups had either succumbed because of growth of the injected tumor cells, or exhibited tumor growth in all peripheral lymphoid organs with meta-static growth also in the liver (data not shown). Thus, the significant differences in tumor growth that were noted at days 9 and 11 after injection of lymphoma cells did not persist after the *qigong* treatments had been stopped. These results suggest that although tumor growth may have been slowed during the time the *qigong* treatments were being administered, progressive tumor growth occurred in all groups after cessation of treatment.

## Study 2

**Methods.** Study 2 was conducted in March and April 2000 in an attempt to replicate the findings of the first study. Except for the modifications indicated below, the basic design was the same as that used in study 1. Sixty (60) mice

TABLE 1. COMPARISON OF GROUP MEANS ON DAY 9 AND DAY 11 (STUDY 1)

Time (days)	Organ	Treatment group			Statistical analysis	
		Control	Qigong	Sham	F test	p
9	LN	(n = 8) 1.39 <sup>a</sup> (0.23)	(n = 9) 1.10 <sup>a,b</sup> (0.25)	(n = 9) 1.36 <sup>b</sup> (0.28)	3.401	0.05
	Spleen	1.72 (0.26)	1.53 (0.37)	1.79 (0.36)	1.460	0.25
11	LN	(n = 13) 2.44 <sup>a</sup> (0.38)	(n = 11) 1.90 <sup>a</sup> (0.31)	(n = 11) 2.20 (0.43)	6.138	0.006
	Spleen	3.31 <sup>a,b</sup> (0.39)	2.40 <sup>a</sup> (0.44)	2.69 <sup>b</sup> (0.58)	11.840	<0.001

Numbers in parentheses are standard deviations.

SNK and Duncan tests were applied in *posthoc* tests of group differences: identical superscript letters (<sup>a</sup> or <sup>b</sup>) indicate significant differences between the groups ( $p < 0.05$ ). If both columns have the same superscript letter (say <sup>a</sup>), this implies they are significantly different from each other at  $p < 0.05$ .

LN, lymph node.

were used in this study, and were randomly assigned to the three groups (20 each) as described previously. All mice were injected with lymphoma cells 4 hours after the first *qigong* treatment. Because positive but modest results were obtained in study 1 using treatments on alternating days, it was decided by the *qigong* healer to increase treatment frequency in order to possibly optimize any effects of *qigong* on tumor growth.

During this study, the *qigong* healer purportedly emitted external Qi to the mice for 10 minutes per treatment, but gave the treatment on a daily basis, including weekends. Mice were routinely housed 5 per cage, but were temporarily housed 10 per cage during the treatment interval for all three groups. This procedural modification was implemented so that the *qigong* healer could concentrate his attention to a single cage rather than two cages per treatment as was done in the first study. In this trial, half the mice from each group were sacrificed on day 10 after 10 treatments (including the preinjection treatment session), and the remaining mice were sacrificed on day 13 after 13 treatments (including the preinjection treatment session).

*Results.* Table 2 presents the results of day 10 and day 13 tumor-growth assays. The pattern of results observed in study 1 was also observed in study 2, in that mice in the *qigong*-treated group exhibited less tumor growth in LN and the spleen, compared to the sham-treated and no-treatment groups. However, in

study 2, none of these differences achieved statistical significance.

## DISCUSSION

This is a randomized dual-blind trial to examine the effect of *qigong* therapy on lymphoma growth in mice. The term dual-blind is used here instead of double-blind to reflect the fact that the providers of active and sham interventions cannot be masked, but that the assessor is masked. This terminology follows a convention suggested by Caspi et al. (2000). In two separate trials, mice injected with lymphoma cells and given *qigong* treatment showed less tumor growth compared to a sham-treatment group and a no-treatment control group. In the first study, results achieved statistical significance, while in the second study the results did not. The lack of significance in the second study is particularly disconcerting, because in that study twice as many *qigong* treatments were administered during the observation period. Despite this fact, the differences in tumor growth between the *qigong*-treated group and the control group were actually smaller than in the first study, in which fewer *qigong* treatments were given. In addition, standard deviations in study 2 were generally much larger than in study 1. The basis for this increased variability is unclear, but may be because, in part, of differences in the tumor cell preparations that were used in each study or to the effectiveness of the *qigong* treatment in each study.

TABLE 2. COMPARISON OF GROUP MEANS BY DAY 10 AND 13 (STUDY 2)

Time (days)	Organ	Treatment group			Statistical analysis	
		Control (n = 10)	Qigong (n = 10)	Sham (n = 10)	F	P
10	LN	2.10 (0.39)	1.96 (0.52)	2.36 (0.47)	1.925	0.17
	Spleen	2.35 (0.38)	1.94 (0.46)	2.20 (0.34)	2.629	0.09
13	LN	4.53 (1.00)	4.27 (0.81)	4.68 (0.27)	0.731	0.49
	Spleen	3.87 (0.70)	3.51 (0.34)	3.91 (0.56)	1.617	0.22

Numbers in parentheses are standard deviations.  
LN, lymph node.

The traditional Chinese healing practice called *qigong* is presumed to operate on the basis of the movement and balancing of a subtle energy, called *qi*, which permeates the body, as well as the Universe. Scientific evidence for its effectiveness as a healing modality is very limited and often methodologically flawed. A frequently suggested alternative explanation for its observed effect on patient is the placebo effect, or healing through suggestibility. Therefore, a study of *qigong* effects on animals is especially useful for determining the existence of this subtle human energy.

In study 1, we found that LNs from mice in the *qigong*-treated group were significantly smaller than those from mice in either the control group or the sham treatment group ( $p < 0.05$ ), suggesting that there be less tumor growth in the *qigong*-treated mice. However, careful analysis of the methods used in study 1 for the handling and transportation of mice for their treatments revealed differences among the groups. For example, mice in the no-treatment control group were not brought to the site where the *qigong* and sham treatments were administered. Instead, they remained in the animal facility during these intervals. This probably resulted in the mice receiving the *qigong* treatment also experiencing the stressor of transportation while the control animals did not receive this stressor. Some of the apparent *qigong* effects could therefore be attributed to this stressor and not to the *qigong*. The design of study 2 was modified to address these differences so that the mice in all groups were handled and transported in an identical fashion. In study 2, there were no significant differences among the three groups. The data of study 1 combined with study 2 seem to suggest that the "*qigong*" effects seen in the first study were at least partly related to the stressor of transportation. While it is possible that the *qigong* treatment had an effect on this tumor model in the mice studied, a more conservative interpretation of the data does not support this conclusion. Nevertheless, the fact that in all cases the *qigong*-treated animals had lower tumor mass is intriguing and merits further investigation.

Because subtle energy healing techniques, including *qigong*, all involve the emission of this putative energy by humans, the stability of the phenomenon is constrained by variations in such factors as mood or fatigue between and within practitioners, in contrast to highly standardized and reliable conventional medical therapies. Such factors as an unfamiliar environment, the presence of skeptical observers, and even the attitude of the (blinded) investigator may affect the possible results, even within the same study (Feng, 1994; Schlitz, 1998). In a previous study involving the same *qigong* healer of this study (Chen et al., 1997), the inhibition of liver cancer growth was reported to be as high as 79% after four 10-minute treatments in a solid tumor model. In contrast, the marginal inhibition of tumor growth seen in the *qigong*-treated groups in the experiments described herein may also be, in part, because of the type and growth behavior of the tumor cells being used. In the liver cancer model, the tumor mass is localized at the site of implantation with little metastasis of tumor cells away from the subcutaneous site. In contrast, in the lymphoma model, the tumor cells are injected intravenously and have the ability to metastasize to all of the lymphoid tissues, as well as other organs, such as the liver and lungs. Thus, it is possible that the lymphoma model presents a much more stringent test of the efficacy of external *qigong* treatment on tumor growth.

In summary, in two separate studies involving *qigong* treatment of an inbred strain of mice injected with syngeneic lymphoma cells, a pattern of results emerged that suggested that *qigong* treatment retarded growth of the cancer cells when compared to a sham *qigong*-treatment and a no-treatment control group. In one study, the results achieved statistical significance, while in the other study they did not. Thus, while certainly not conclusive at this point, results suggested that the *qigong* treatment had a tumor inhibitory effect in this model, indicating the need for additional studies to determine the parameters and limitations of this traditional Chinese therapeutic technique.

## ACKNOWLEDGMENT

This study was partially supported by National Institute of Health grant U24-HD32994 (principal investigator: Samuel C. Shiflett, Ph.D.) and a grant from a private source.

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