Experimental

CULTURED HUMAN BRAIN TUMOR CELLS DO NOT RESPOND TO JOHREI TREATMENT

Ryan Taft; Luz Nieto; Tri Luu; Alyson Pennucci; Dan Moore, Ph.D. & Garret Yount, Ph.D.

ABSTRACT

Patients faced with grim diagnoses, such as glioblastoma multiforme (GBM), an incurable brain tumor for which standard therapy is debilitating, are increasingly using alternative healing practices that purportedly involve the manipulation of some form of healing energy. To evaluate the possibility that such practices can affect biological targets, adherents of one alternative healing practice, Johrei, were invited to treat GBM cells in culture. Such *in vitro* models effectively eliminate the factor of psychological cueing. We conducted 40 experiments involving Johrei treatment and 28 control experiments in which a person with no training as a healer substituted for the practitioners. Each experiment incorporated strict randomization and blinding techniques. Johrei treatments were delivered from an average distance of 30 cm. We used time-lapse microscopy to investigate the rates of cell death and proliferation before, during, and after treatment. We found no significant differences between Johrei and control experiments for either tumor cell death or proliferation.

KEYWORDS: Johrei, cell culture, energy medicine, time-lapse microscopy

INTRODUCTION

In records spanning from the earliest legends to modern popular culture, continuous allusions can be found to the ability of individuals to influence the health of others without physically touching them.[1] Such allusions are central to alternative healing practices that purportedly involve the manipulation, or "channeling" of some form of scientifically undocumented energy. While medical professionals have generally regarded such claims as irrational, it becomes the scientific community's responsibility to scrutinize these assertions as more people turn to these practices for what they believe is therapeutic treatment. Provocative results from recent studies evaluating a Japanese healing practice, Johrei, indicate that the efficacy of this alternative healing modality can be demonstrated under strict experimental conditions. Adherents claim that Johrei is a method of "channeling divine light" to a patient which results in "spiritual purification." Additionally, Johrei emphasizes a of type of flower arrangement (Ikebana) and a variety of organic farming. College students trained in Johrei displayed an immune profile consistent with a possible stress reduction relationship.[2] Additionally, a line of parallel research suggested a beneficial effect of Johrei on mood.[3]

Whether the apparent efficacy of such healing practices is due to processes internal or external to the subject is not clear, however. To this end, we were interested in eliminating the mind of the subject as a confounding variable. In order to test the possible direct effect of Johrei treatment we asked 10 experienced Johrei practitioners to direct "healing intention" toward cultured human cancer cells under direct microscopic observation. For these experiments we chose a cell model of glioblastoma multiforme (GBM), the most malignant form of human brain cancer. Despite over 30 years of research investigating novel therapeutics for this illness, only 29% of patients diagnosed with GBM survive one year or more.[4] Because of the grim prognosis of this disease, and because its standard treatments are suboptimal,[5] patients often turn to alternative therapies. The interest of GBM patients in noninvasive alternatives is reflected by an ongoing federally-funded clinical trial at the California Pacific Medical Center Research Institute investigating the efficacy of distant healing modalities, including Johrei, against GBM.

MATERIALS AND METHODS

Overall Study Design

For each session Johrei practitioners were asked to direct healing intention toward cell cultures. Johrei practitioners participated in teams of two, alternating every half hour such that a total of 4 hours of Johrei treatment was delivered. Data acquired from each of two active side-by-side time-lapse microscopes were considered an independent experiment, such that having five practitioner teams participate in four independent sessions each resulted in a total of 40 Johrei experiments. We interspersed these with 28 experiments in which no healing intervention was delivered but a person sat in front of the cell cultures to control for the close proximity of a human body. Johrei or control treatments were initiated after 4 hours of baseline data had been collected. The observation period extended for a total of 12 hours. We quantified tumor cell death and proliferation throughout the observation period.

Randomization and Blinding Procedures

Experiments were conducted with blinding applied to each of the scientists and the biostatistician involved, following previously reported methods.[6] Briefly, the experimental protocol was divided among scientists such that those responsible for preparation of the cell cultures, data acquisition, data analysis, Johrei intervention monitoring, and statistical analysis were all blind to each other's activities until data analysis was complete. All data and blinding codes were sent to a code keeper at an outside institution for independent verification. Throughout cell culture preparation we assigned each sample randomly to a position in the incubator. A computer program written for this purpose (Microsoft Visual Basic 5.0) used a pseudo-random number generator to assign plates to positions on a grid marked on the incubator shelf. Each plate had equal likelihood of assignment to any incubator position. This allowed us to rule out the possibility of incubator position as possible cause of any observed effect.

Cell Culture

As the target of healing intentionality in these studies, we used a human cell line (SF 188 GBM) derived from a brain tumor biopsy specimen from a patient with GBM. This cell culture model is used widely and is responsive to conventional therapies. The model can demonstrate both positive and negative responses (cell proliferation and cell death), depending on the treatment. SF 188 GBM cells were grown in RPMI media supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin. Cells were passaged at confluence using trypsinization and were expanded to a large population size, aliquoted, and frozen viably for long-term storage. A fresh aliquot was thawed at the start of each experimental trial to ensure uniformity in the genetic profile of the target cells throughout the investigation. Cells were plated at a density of 50,000 cells per well in six-well culture plates, which maintained cells at 25-45% confluence per well throughout the experiments. Prior to each experiment cells were allowed to grow uninterrupted for 46 hours in a humidified incubator maintained at 37°C and 5% CO_2.

Time-Lapse Microscopy

For each experimental session, the cell cultures were transferred from the incubator to a time-lapse microscope (Axiovert 100; Carl Zeiss AG: Oberkochen, Germany) equipped with an on-stage environmental chamber at the University of California, San Francisco Comprehensive Cancer Center. Cell cultures were maintained at routine incubation settings (37°C, 5% CO_2) and optimum humidity. Temperature and CO_2 concentration were independently controlled using digital controlling units (Carl Zeiss AG: Oberkochen, Germany). Cells were visualized at 200x magnification. Two sets of phase contrast images from each well were taken in 300 second intervals using a COHU RS-170 monochrome CCD camera. An Openlab software (Improvision, Lexington, MA) automation drove the camera and stage movements, and compiled the acquired phase images. Images were subsequently processed as Quicktime movies using Openlab. Each time-lapse experiment ran for 12 hours.

JOHREI INTERVENTION

Johrei practitioners were selected based on experience by the Center for the Science of Life, a Johrei organizational body in the United States. Ten Johrei practitioners with an average of 30 years experience participated in the experiments in teams of two. A total of 4 hours of Johrei treatment was administered during each experiment. Each practitioner treated the cells for a total of 2 hours per experiment, switching every half hour with the team member. Treatment began with one of the two practitioners being seated in front of one of the two time-lapse microscopes and raising one hand toward the cellular target. The cellular targets in the second microscope served as indirect targets (20 cm away from the direct targets). One hand remained raised toward the cell cultures for the duration of any given Johrei treatment. Johrei treatments were delivered from a distance of 5 - 40 cm (average = 30 cm). Distance ranges were the result of individual practitioner preferences. All practitioners asserted that they believed treatment delivered from any given distance would be effective. Practitioners were supervised by a scientific monitor to verify that they did not tamper with the experimental devices or cell cultures.

CONTROL INTERVENTION

Laboratory personnel with no training in a healing practice participated in control experiments to mimic the presence of a human body in close proximity to the cells under observation. These experiments were included to address whether any observed effect was simply due to the non-specific effects of human presence (e.g. body heat). Control subjects were matched to Johrei practitioners for approximate weight and height. Identical to the protocol for Johrei practitioners, control subjects sat directly in front of one of the two time-lapse microscopes. However, only one control individual was used per experiment, unlike the teams of two used for Johrei intervention. Control subjects sat in front of the cells for a total of four hours. Control subjects were asked not to direct any conscious attention toward the cells.

TIME-LAPSE MICROSCOPY DATA ANALYSIS

Every cell was identified and numbered in the initial screen of the Quicktime movies generated from each time-lapse experiment. All numbered cells and

their progeny were then tracked for the duration of their onscreen viability. Each cell's life events were charted, using a modified version of a previously described cell pedigree system.[7] To accurately assess our primary outcome measures we identified the following events for each cell: 1) rounding time, 2) division time, 3) time of cell death, 4) time of apoptotic morphological characteristics including blebbing, spiking, and cytoplasmic swelling as described previously,[8] 5) any morphological changes not already identified, and 6) migration out of the microscopic field. Cells identified as dead at the start of the video or that entered the microscopic field after the initial frame were not included in this analysis. Cataloged data was entered into a Microsoft Excel spreadsheet for further analysis. Deaths and divisions occurring per hour were denoted as well as all parameters mentioned above.

Statistical Analysis

Statistical analysis was based on a model which allows a cell to be engaged in any one of four activities at any time: 1) division, resulting in an additional cell (division), 2) death, resulting in the loss of a cell (death), 3) movement out of the microscopic field (emigration), or 4) the cell can remain unchanged. Activities 3 and 4 were quantified in order to accurately determine the rates of division and death and were not used as reliable hypothesis testing measures. Under this model three transition probabilities plus the total number of cells at a previous time determine the number of cells at a future time. The expected number of cells at time t, $N(t)$, is given by the equation

$$N(t) = N(t - 1)\ \exp(\lambda(t) - \mu(t) - v(t))] \qquad (1)$$

where $\lambda(t)$, $\mu(t)$ and $v(t)$ are the transition probabilities for division, death and emigration, respectively, at time t. We estimated the transition probabilities in one-hour time blocks. For example, the estimate for $\lambda(t)$ is

$$\lambda(t) = [\ln(N(t) + div(t)) - \ln(N(t - 1))] \qquad (2)$$

where $div(t)$ is the number of divisions during $(t - 1, t)$. A similar equation was used for estimating cell death transition probabilities at each hour.

Table I

*Average number of events per experiment per hour**

	Johrei (N=40)				Controls (N=28)			
Time	**N (t)**	**div(t)**	**d(t)**	**e(t)**	**N (t)**	**div(t)**	**d(t)**	**e(t)**
0	27.28				36.75			
1	26.95	0.93	0.68	0.15	36.32	0.75	0.68	0.00
2	28.03	1.80	0.40	0.33	37.04	1.39	0.32	0.36
3	28.68	1.20	0.30	0.25	38.04	1.79	0.29	0.50
4	29.10	1.13	0.33	0.38	38.89	1.64	0.21	0.57
5	29.28	1.03	0.55	0.30	39.43	1.39	0.32	0.54
6	28.98	0.68	0.53	0.45	39.46	1.14	0.32	0.79
7	29.08	0.93	0.45	0.38	39.46	1.00	0.25	0.75
8	29.40	1.15	0.45	0.38	39.96	1.50	0.21	0.79
9	29.65	0.98	0.35	0.38	40.25	1.21	0.21	0.71
10	29.73	0.80	0.40	0.33	40.25	1.21	0.32	0.89
11	29.93	0.63	0.20	0.23	40.00	0.86	0.43	0.68
12	29.95	0.98	0.53	0.43	40.00	1.00	0.50	0.50

*(N = number of cells, div = divisions, d = deaths, e = emigrations)

Statistical analysis focused on two types of comparisons: comparisons within the Johrei treatment experiments, where division and death rates (transition probabilities) pre-treatment were compared with those during and post-treatment; and comparisons between Johrei and control experiments at similar times. The primary outcomes were division and death rates during a 4-hour period when the Johrei practitioner was directing "healing intention" toward the cells or a control subject was present. None of the one hour transition probabilities were normally distributed, so nonparametric tests were used for all statistical comparisons.

RESULTS

Conducting 40 experiments involving Johrei practitioners allowed us to document the behavior of 2,768 cells exposed to healing treatments (~69 cells/experiment). We also documented the behavior of 1,827 cells in 28 control experiments (~65 cells/experiment). Hourly mean counts are shown in Table I. Caution should be used in comparing means in Table I because the numbers

Table II
Estimated Mean Transition Rates

Time	Jolirei (N=40) division(λ)	Jolirei (N=40) death(μ)	Control (N=28) division(λ)	Control (N=28) death(μ)
0 - 1	0.0304	0.0231	0.0225	0.0201
1 - 2	0.0642	0.0282	0.0408	0.0079
2 - 3	0.0450	0.0131	0.0509	0.0082
3 - 4	0.0368	0.0100	0.0470	0.0043
4 - 5	0.0395	0.0117	0.0356	0.0087
5 - 6	0.0208	0.0197	0.0266	0.0095
6 - 7	0.0292	0.0236	0.0246	0.0069
7 - 8	0.0378	0.0214	0.0342	0.0059
8 - 9	0.0298	0.0266	0.0271	0.0065
9 - 10	0.0289	0.0232	0.0290	0.0087
10 - 11	0.0169	0.0366	0.0200	0.0108
11 - 12	0.0266	0.0095	0.0216	0.0149

of events depend on the numbers of events at preceding times, and the data are not normally distributed (i.e. many of the individual counts are zero). However, it is evident that the total number of cells observed (denoted by *N(t)* in Table I), initially and at subsequent times, is greater in the control experiments than in the Johrei experiments. The probability that the observed difference in the distribution of the initial numbers of cells plated in Johrei vs. controls is due to chance is $p < 0.005$ (Mann-Whitney comparison of ranks of the numbers). Based on the fact that plating procedures for both the control and Johrei interventions were identical this difference was likely due to differences in the number of cells in the microscopic fields chosen. To adjust for field differences a more accurate assessment of cellular activity was accomplished using transition rates rather than the absolute number of events of different types (i.e. divisions and deaths).

Transition rates are shown in Table II and plotted in Figures 1 and 2. Rates of division rose during the first two hours (pre-treatment), reaching a peak during the third hour, and then declined slowly afterward. Note that symbols in the figure appear both on the x-axis and within the graph because many of the individual counts are zero. Nonparametric tests found the trend statisti-

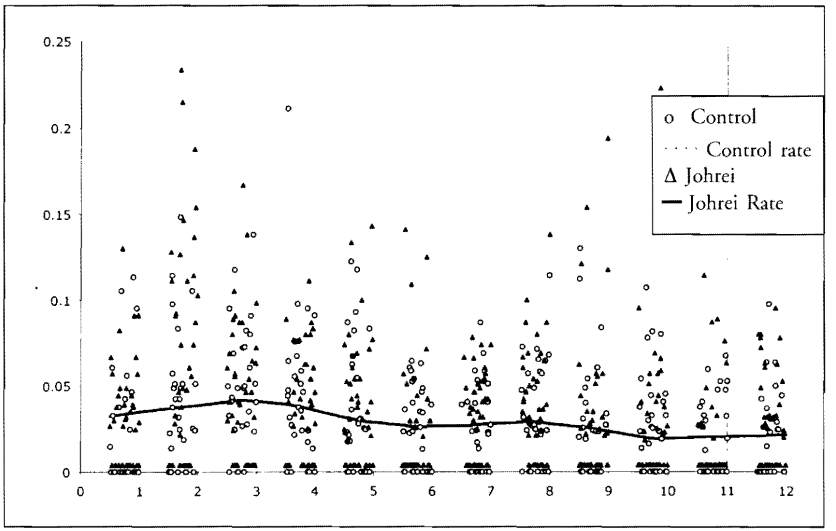

Figure 1. *Cell divisions of cultured human GBM cells per hour. Experimental treatments (Johrei or control) occurred during hours 4-8. Rates are depicted as individual points and smoothed lines (locally weighted least squared fit).*

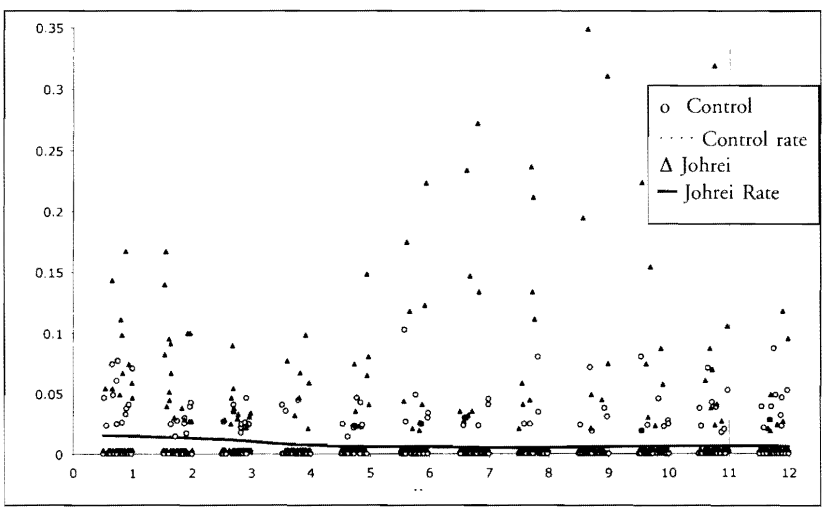

Figure 2. *Cell deaths of cultured human GBM cells per hour. Experimental treatments (Johrei or control) occurred during hours 4-8. Rates are depicted as individual points and smoothed lines (locally weighted least squared fit).*

Table III
Time period analysis comparing Johrei and control experiments

Comparison	p-values from Mann-Whitney test divisions	deaths
within Johrei experiments		
pre vs. during vs. post treatment	0.0001	0.15
pre vs. during treatment	0.03	0.08
during vs. post treatment	0.02	0.82
Johrei vs. control	0.82	0.67
pre treatment	0.37	0.29
during treatment	0.91	().89
post treatment	0.38	0.60

ANOVA for time block, Johrei & interaction	Variance	p-values
time	0.0329	<.0001
Johrei vs. control	0.0014	0.31
time x treatment interaction	0.0007	0.48

cally significant in both Johrei and control experiments (p = 0.0001 for Johrei and p = 0.005 for control based on the Kruskal-Wallis test). We used analysis of variance to test whether the time period trend was the same for Johrei and control. The hypothesis of no difference was accepted (p < 0.31, Table III).

There were no significant differences between Johrei and control cell cultures for division or deaths during any time period (Johrei vs. control, Table III). We observed a high degree of variation during the 5-10 hour time period relative to the other time periods, but the means show no effect of Johrei treatment.

DISCUSSION

We found no significant difference in cell death or proliferation in cultures of human tumor cells treated by Johrei practitioners. Time-lapse microscopy allowed us to assess these outcome measures before, during and after Johrei

treatments. These negative results are consistent with recent studies evaluating a healing practice similar to Johrei, external Qigong, whose practitioners claim to manipulate an unknown energy to treat patients. In one well-designed study, Shah and colleagues tested whether a Qigong practitioner with 18 years experience could affect the cell growth of four different cancer lines *in vitro*.[9] This group found no significant difference between treated or control cell cultures. Importantly, a recent report suggested that after repeated trials in both the United States and China, no reproducible effects of external Qigong on cultured cells could be observed.[10] These negative results are bolstered by analyses indicating that the Asian Qigong literature reporting positive *in vitro* effects may be questionable.[11,12]

The potential therapeutic value of Johrei and other healing practices may not be diminished if future studies verify that no direct effect can be measured using *in vitro* models. Cell cultures may present a limited model, lacking features of an intact system as a target of "healing intention." For example, exploration of other experimental models that include human or animal subjects may be warranted. Additionally, it is possible that healing practices like Johrei may affect patients' health through the interwoven network of central nervous and endocrine system processes involved in modulation of the immune system in response to psychological states.[13,14] Indeed, there is a growing body of research suggesting that interventions which alter mental or behavioral patterns are capable of effecting substantial physiologic change. For example, two recent studies have suggested that mindfulness-based meditation may be associated with enhanced quality of life, decreased symptoms of stress, changes in cancer-related cytokine production and significant increases in left-sided anterior activation of the brain, a pattern previously associated with positive affect.[15-17] Well controlled studies of internal Qigong, a practice involving meditation and movement, may benefit those suffering from fibromyalgia, hypertension, and type II diabetes.[18-21] Indeed, the weight of evidence linking physiologic changes and psychological states (e.g. stress[22,23]) impacts aspects of both alternative and conventional medicine. Examples range from clinical "bedside manner" to evangelical mass healings. Elements of healing practices such as Johrei necessarily include these elements as part of their treatment. A better understanding of such psychophysiological links may allow the development of therapeutic strategies to maximize their benefit.

• • •

CORRESPONDENCE: Garret Yount, Ph.D. • California Pacific Medical Center Research Institute • 475 Brannan Street, Suite 220 • San Francisco, CA 94107 • Email: yountg@sutterhealth.org

ACKNOWLEDGEMENTS: This work was funded in part by the Rockefeller-Samueli Center for Research in Mind-Body Energy, The Samueli Institute for Information Biology, The Center for the Science of Life, and grant R01 AT000516 from the National Center for Complementary and Alternative Medicine.

REFERENCES & NOTES

1. M. Schlitz, Intentionality in Healing: Mapping the Integration of Body, Mind and Spirit, *Alternative Therapies in Health and Medicine* **1** (1995), pp. 119-120.
2. A. Naito, T. M. Laidlaw, D. C. Henderson, L. Farahani, P. Dwivedi & J. H. Gruzelier, The Impact of Self-Hypnosis and Johrei on Lymphocyte Subpopulations at Exam Time: A Controlled Study, *Brain Research Bulletin* **62** (2001), pp. 241-245.
3. T. M. Laidlaw, M. Naito, P. Dwivedi, N. A. Enzor, C. E. Brincat & J. H. Gruzelier, Mood Changes After Self-Hypnosis and Johrei Prior to Exams, Contemporary Hypnosis **20** (2003), pp. 25-40.
4. CBTRUS, Statistical Report: Primary Brain Tumors in the United States, 1995-1999. Published by the Central Brain Tumor Registry of the United States (2002).
5. E. A. Maher, F. B. Furnari, R. M. Bachoo, D. H. Rowitch, D. N. Louis, W. K. Cavenee & R. A. DePinho, Malignant Glioma: Genetics and Biology of a Grave Matter, *Genes and Development* **15** (2001), pp. 1311-1333.
6. M. Schlitz, D. Radin, B. F. Malle, S. Schmidt, J. Utts & G. L. Yount, Distant Healing Intention: Definitions and Evolving Guidelines for Laboratory Studies, *Alternative Therapies in Health and Medicine* **9** (2003), pp. A31-43.
7. H. B. Forrester, C. A. Vidair, N. Albright, C. C. Ling & W. C. Dewey: Using Computerized Video Time Lapse for Quantifying Cell Death of X-irradiated Rat Embryo Cells Transfected withi c-myc or c-Ha-ras, *Cancer Research* **59** (1999), pp. 931-939.
8. B. Enidlich, I. R. Radford, H. B. Forrester & W. C. Dewey, Computerized Video Time-Lapse Microscopy Studies of Ionizing Radiation-Induced Rapid-Interphase and Mitosis-Related Apoptosis in Lymphoid Cells, *Radiation Research* **153** (2000), pp. 36-48.
9. S. Shah, A. T. Ogden, C. M. Pettker, A. Raffo, S. Itescti & M. C. Oz, A Study of the Effect of Energy Healing on *In Vitro* Tumor Cell Proliferation, *Journal of Alternative and Complementary Medicine* **5** (1999), pp. 359-365.
10. G. Yount, J. Solfvin, D. Moore, M. Schlitz, M. Reading, K. Aldape & Y. Qian, *In Vitro* Test of External Qigong, *BMC Complementary and Alternative Medicine* **5** (2004), p. 359-365.
11. W. B. Jonas & C. C. Crawford, Science and Spiritual Healing: A Critical Review of Spiritual Healing, "Energy" Medicine and Intentionality, *Alternative Therapies in Health and Medicine* **9** (2003), pp. 56-61.
12. H. L. Zhang, *Revealing the True Face of Qigong: Corrections and Clarification of Origin* (China Social Science Publication Company, Beijing, P. R. China, 1996).
13. C. B. Pert, H. E. Dreher, M. R. Ruff, The Psychosomatic Network: Foundations of Mind-Body Medicine, *Alternative Therapies in Health and Medicine* **4** (1998), pp. 30-41.

14. J. K. Kiecolt-Glaser, L. McGuire, T. F. Robles & R. Glaser, Psychoneuroimmunology and Psychosomatic Medicine: Back to the Future, *Psychosomatic Medicine* **64** (2002), pp. 15-28.
15. L. E. Carlson, M. Speca, K. D. Patel & E. Goodey, Mindfulness-Based Stress Reduction in Relation to Quality of Life, Mood, Symptoms of Stress and Levels of Cortisol, Dehydroeplandrosterone Sulfate (DHEAS) and Melatonin in Breast and Prostate Cancer Outpatients, *Psychoneuroendocrinology* **29** (2004), pp. 448-474.
16. L. E. Carlson, M. Speca, K. D. Patel & E. Goodey, Mindfulness-Based Stress Reduction in Relation to Quality of Life, Mood, Symptoms of Stress, and Immune Parameters in Breast and Prostate Cancer Outpatients, *Psychosomatic Medicine* **65** (2003), pp. 571-581.
17. R. J. Davidson, J. Kabat-Zinn, J. Schumacher, M. Rosenkranz, D. Muller, S. F. Santorelli, F. Urbanowski, A. Harrington, K. Bonus & J. F. Sheridan, Alterations in Brain and Immune Function Produced by Mindfulness Meditation, *Psychosomatic Medicine* **65** (2003), pp. 564-570.
18. M. S. Lee, H. J. Kim & E. S. Choi, Effects of Qigong on Blood Pressure, High-density Lipoprotein Cholesterol and Other Lipid Levels in Essential Hypertension Patients, *International Journal of Neuroscience* **114** (2004), pp. 777-786.
19. M. S. Lee, H. J. Kim & S. R. Moon, Qigong Reduced Blood Pressure and Catecholamine Levels of Patients with Essential Hypertension, *International Journal of Neuroscience* **11** (2003), pp. 1691-1701.
20. J. A. Astin, B. M. Berman, B. Bausell, W. L. Lee, M. Hochber & K. L. Forys, The Efficacy of Mindfulness Meditation plus Qigong Movement Therapy in the Treatment of Fibromyalgia: A Randomized Controlled Trial, *Journal of Rheumatology* **30** (2001), pp. 2257-2262.
21. M. Iwao, S. Kajiyama, H. Mori & K. Oogaki, Effects of Qigong Walking on Diabetic Patients: A Pilot Study, *Journal of Alternative and Complementary Medicine* **5** (1999), pp. 353-358.
22. J. E. Platt, X. He, D. Tang, J. Slater & M. Goldstein, C-fos Expression *In Vivo* in Human Lymphocytes in Response to Stress, *Progress in Neuropsychopharmacoloy, Biology and Psychiatry* **19** (1995), pp. 65-74.
23. R. Glaser, W. P. Lafuse, R. H. Bonneau, C Atkinson & J. K. Klecolt-Glaser, Stress-Associated Modulation of Proto-oncogene Expression in Human Peripheral Blood Leukocytes, *Behavioral Neuroscience* **107** (1993), pp. 525-529.

∞ ∞ ∞