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Original Article

Radiation Response of Cultured Human Cells Is Unaffected by Johrei

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Johrei has been credited with healing thousands from radiation wounds after the Hiroshima and Nagasaki bombs in 1945. This alternative medical therapy is becoming increasingly popular in the United States, as are other Energy Medicine modalities that purport to influence a universal healing energy. Human brain cells were cultured and exposed to increasing doses of ionizing radiation. Experienced Johrei practitioners directed healing intentionality toward the cells for 30 min from a distance of 20 cm and the fate of the cells was observed by computerized time-lapse microscopy. Cell death and cell divisions were tallied every 30 min before, during and after Johrei treatment for a total of 22.5 h. An equal number of control experiments were conducted in which cells were irradiated but did not receive Johrei treatment. Samples were assigned to treatment conditions randomly and data analysis was conducted in a blinded fashion. Radiation exposure decreased the rate of cell division (cell cycle arrest) in a dose-dependent manner. Division rates were estimated for each 30 min and averaged over 8 independent experiments (4 control and 4 with Johrei treatment) for each of 4 doses of X-rays (0, 2, 4 and 8 Gy). Because few cell deaths were observed, pooled data from the entire observation period were used to estimate death rates. Analysis of variance did not reveal any significant differences on division rate or death rate between treatment groups. Only radiation dose was statistically significant. We found no indication that the radiation response of cultured cells is affected by Johrei treatment.

Keywords: biofield therapy – cell culture – energy medicine – spiritual healing – time-lapse microscopy

Background

Johrei (pronounced Jo-ray) was founded by Mokichi Okada in Japan in 1935; 'Joh' means purify and 'Rei' means soul, spirit or ghost. It is considered by some to be a manifestation of divine energy that can be channeled through one individual to another for healing. In a Johrei healing session, divine energy is directed from a practitioner's body, through his hands, to various parts of a recipient's body. Three principles form the pillars of Johrei philosophy as follows: (i) that divine energy can be used to heal, (ii) that surrounding one's self with beauty and art perpetuates fulfillment in life, and (iii) that natural farming allows for more wholesome growth of our bodies and our divine energy (1). At this time, an estimated 5 million people practice Johrei worldwide, according to Johrei organizational bodies in the Unites States. The history of Johrei includes compelling case reports maintained at the National Archives in Washington, DC, documenting recovery with Johrei treatment from deadly exposure to ionizing radiation released from the atomic bomb in Hiroshima and Nagasaki (2). In this study, we developed a cell culture model of radiation exposure using normal human brain cells. Experienced Johrei practitioners came to the laboratory and channeled divine energy toward the cells with the intention of facilitating recovery from the toxic effects of radiation.

Normal human cells sense radiation-induced damage to their genetic material and stop their life cycle, allowing time for DNA repair processes to occur before the cell divides. The suppression of cell division is proportional to the degree of radiation-induced DNA damage and is thought to allow time for DNA repair so that mutations in the DNA are not 'hard-wired' and passed on to progeny (3). We assessed whether Johrei treatment could facilitate the recovery of the

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cells from radiation exposure in the form of increased division rates compared to untreated control cells. We also assessed whether Johrei treatment altered the degree of cell death following irradiation.

Methods

Summary of Experimental Design

Primary cultures of normal human glial cells were exposed to increasing doses of X-rays in a clinical setting and examined by computerized time-lapse microscopy. Two Johrei practitioners participated in two independent experiments each ($n = 4$). An equal number of control experiments were performed in which nobody entered the treatment room during the treatment period (no treatment). The potential impact of subsequent Johrei treatment was assessed by comparing cell proliferation and cell death with the same measures in control experiments. Time-lapse data were acquired for 4 h before treatment, throughout the Johrei treatment and for 18 h following treatment (total of 22.5 h). Preparation of the cell cultures, data acquisition and data analysis were divided among scientists. Experiments were conducted with blinding applied to each of the scientists based on previously reported methods (4), and results remained blinded until the analysis was complete.

Cell Culture and Irradiation

Primary human mixed glial cultures were isolated by established methods (5) and were confirmed to be glia by uniform staining with an anti-glial fibrillary acid protein antibody (data not shown). Radiation treatment was delivered to cell suspensions before plating using an Oldelft Therapax 150 X-ray machine at a dose rate of 85.2 cGy min^{-1} with full backscatter. Fresh aliquots of cryogenically preserved cells (both irradiated and non-irradiated) were thawed at the start of each experiment to ensure uniformity throughout the experiments. Cells were cultured in Astrocyte Basal Medium (Biowhittaker, Inc., East Rutherford, NJ) with 20 ng ml^{-1} human recombinant epidermal growth factor, 25 ng ml^{-1} insulin, 25 ng ml^{-1} progesterone, 50 ng ml^{-1} transferrin, 50 ng ml^{-1} gentamicin, 50 ng ml^{-1} amphotericin and 10% fetal bovine serum. For each experiment, cells were seeded into four wells of a six-well plate (Falcon, Franklin Lakes, NJ) at a density of 20 000 cells per well: three wells contained cells that had been exposed to X-rays at 2, 4 or 8 Gy; the fourth culture had not been irradiated (0 Gy). To allow for the initiation of DNA repair processes, cells were grown uninterrupted for ~24 h before the start of each experiment in a humidified incubator maintained at 37°C and 5% CO_2. The location of each culture was randomly assigned to wells in the six-well plate using an online pseudorandom number generator (http://www.random.org/nform.html).

Johrei Treatment

The Center for the Science of Life selected two Johrei practitioners for participation in the experiments. Both practitioners had more than 17 years experience treating people with Johrei. Healing treatments were administered for a total of 30 min with an upraised hand directed toward the incubation chamber (see Fig. 1). The plexiglass wall (4 mm thickness) of the incubation chamber insured that the practitioners' hands remained at least 20 cm away from the cultures at all times. Standard mental procedures were followed by both practitioners to minimize variability in the mental states among practitioners. The mental cues can be summarized briefly as follows: (i) establishing a connection to the divine, (ii) consciously relaxing the body and mind, (iii) visualizing healing energy traveling through the upraised hand and penetrating the cellular target, (iv) taking enjoyment in participating in the experiment and (v) maintaining a feeling of gratitude. Johrei treatment was initiated after 4 h of baseline data were collected.

Computerized Time-Lapse Microscopy

At the start of each experiment, cell cultures were transferred from the incubator to a time-lapse microscope equipped with a heated stage and incubation chamber (Axiovert 200; Zeiss, Gottingen, Germany). The incubation chamber maintained optimum environmental conditions (37°C, 5% CO_2) by independent digital control units (Zeiss, Gottingen, Germany). Two sets of phase contrast images from each well were acquired throughout the experiment using a Cohu 2600 Series compact monochrome interline transfer CCD camera, and taken at 300 s intervals. Openlab software automation

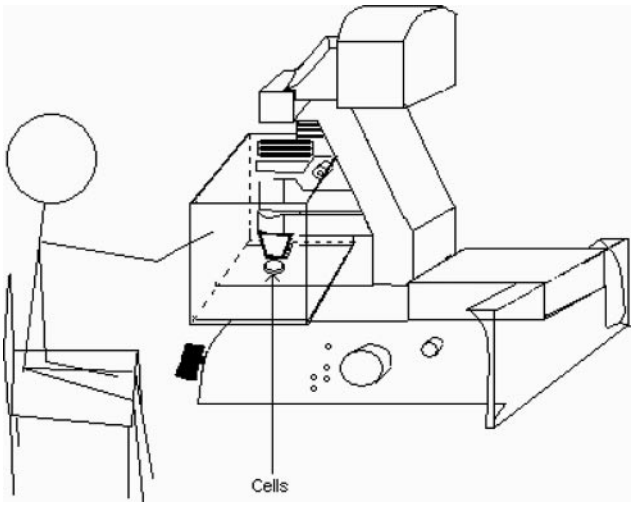

Figure 1. Illustration of Johrei treatments. Treatments involved one Johrei practitioner being seated in front of the time-lapse microscope and raising one hand toward the cellular target. One hand remained raised toward the cell cultures for the duration of treatment. Johrei treatments were delivered from a distance of 20 cm, from outside the plexiglass environmental chamber attached to the microscope.

(Improvision, Lexington, MA) operated the camera, stage movements and compiled the acquired phase images. Images were then processed as Quicktime movies using the above software.

Every cell in the initial microscopic field was identified and numbered. All identified cells and their progeny were tracked for the duration that they were viewed onscreen. Cells that entered the microscopic field after the initial frame were neither included nor were cells identified as dead at the start of the video. Cells divisions and cell deaths were counted for varying numbers of cells over a 22.5 h period, with counts being made every 30 min. We estimated division rates for each 30 min and averaged over eight replicate experiments for each of the four doses (0, 2, 4 and 8 Gy). We estimated death rates over the 22.5 h period because there were very few deaths.

Data Analysis

Statistical analysis was based on a model categorizing a cell as engaging in any one of four activities at any time during the experiment as follows: (i) cell division, resulting in an additional cell introduced into the population (division), (ii) death, resulting in the loss of a cell from the population (death), (iii) movement of a cell out of the microscopic field (emigration) or (iv) the cell remaining unchanged. Three transitional probabilities were possible under this model, plus a total number of cells at a previous time determined the number of cells at a future time. The expected number of cells at time t, $N(t)$, is given by the equation

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

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 30 min time blocks. For example, the estimate for $\lambda(t)$ is

$$\lambda(t) = [\ln(N(t) + \mathrm{div}(t))-\ln(N(t-1))],$$

where $\mathrm{div}(t)$ is the number of divisions during $(t-1t)$. A similar equation was used for estimating death and emigration transition probabilities at each 30 min.

Results

We analyzed the radiation response of 800 cells in four control experiments and 856 cells in four experiments involving Johrei treatment. Table 1 lists the total number of cells observed for each condition.

Cell division rate data for analysis consisted of the averages over eight replicates for each 30 min and each dose and each treatment group (Johrei or control). Cell death rate data for analysis consisted of the averages over eight replicates for each dose and treatment group (one value for the observation period). Analysis of variance was performed to determine whether treatment group and radiation dose had an effect on these outcomes. We also tested division rate over time to see if it was constant. Only radiation dose was statistically significant ($P < 0.0001$). There were no significant differences between Johrei-treated and control cultures for cell division ($P = 0.560$) or cell deaths ($P = 0.456$). Figure 2 depicts the division rates for cells averaged across the replicate experiments.

Table 1. Number of cells examined for each condition

X-ray exposure (Gy)	Experimental condition	
	No treatment (control)	Johrei treatment
0	217	220
2	222	205
4	210	202
8	173	207

Discussion

Methodological Considerations

The failure to observe an effect of Johrei treatment in these experiments is consistent with earlier reports from our laboratory indicating that cultured cells do not respond to Johrei treatment (6,7). The unresponsiveness of the cultured cells may reflect an inadequate modeling of Johrei healing that might be better studied using a clinical model such as a recently published pilot study of family-based Johrei practice evaluating childhood eczema (8). For example, isolation from the intact human organism may strip the cultured cells of crucial signaling mechanisms. A recent study, however, reports growth inhibition of cultured human carcinoma cells following treatment with Ki-energy, a form of Biofield therapy similar to Johrei (9). Thus, further optimization of cell culture models may yield a useful tool for examining direct effects of Johrei and other Biofield therapies. An important next step is the evaluation of long-term outcomes measures following radiation exposure such as colony formation.

Reflections by Practitioners

Further design considerations were identified through dialogue with the participating practitioners. Because the fundamental aim of Johrei healing is to empower the innate ability to restore balance, it was suggested that co-culturing the irradiated cells with healthy cells may allow for a form of 'team work' in which the healthy cells help the stressed cells. Another question raised was whether a spiritual energy dissipates from cells kept alive after the donor is deceased. This could be addressed by evaluating cells collected from living participants, possibly from persons receiving concurrent Johrei treatment. The collection of oral leukocytes by rinsing the mouth with hypertonic salt solution provides a non-invasive method for obtaining fresh cells from living participants (10) and may be useful in this regard.

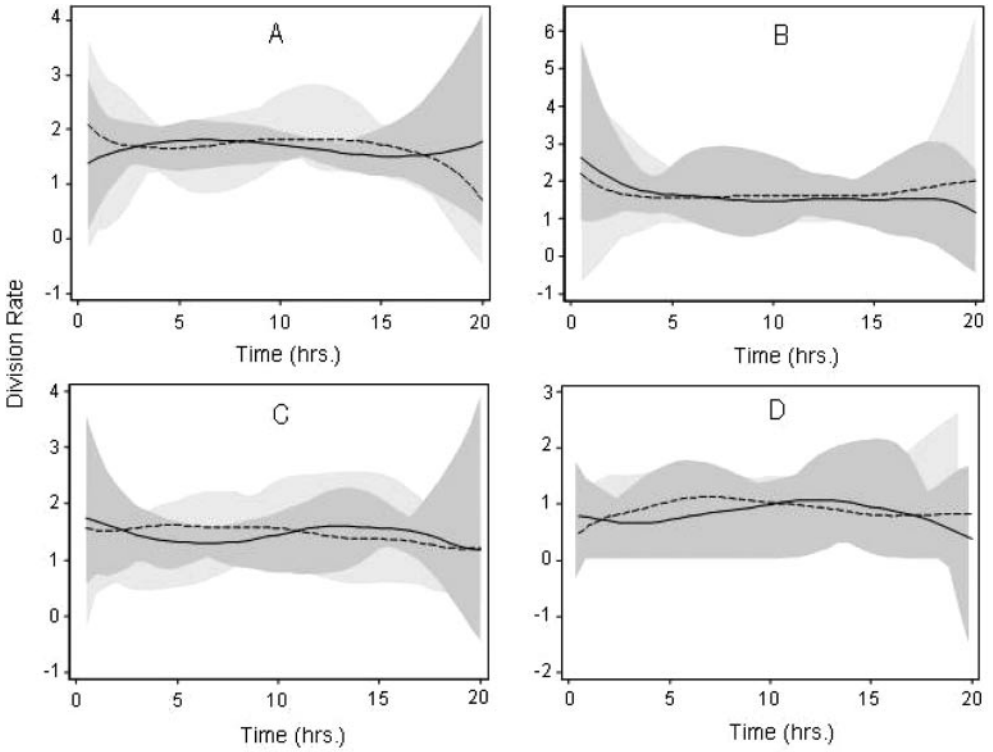

Figure 2. Locally weighted least squares (lowess) smoothed fits to division rate data over time. Lowess was applied to division rate (measured in 30 min increments) for each of eight replicate measurements and the average of the eight for each radiation dose (Panel A: 0 Gy; Panel B: 2 Gy; Panel C: 4 Gy; Panel D: 8 Gy). The dashed line is the smoothed average for control experiments without healing treatments; the solid line is for experiments involving Johrei treatments. The darker shaded region shows the range of smoothed replicate rates for controls; the lighter shaded region is for Johrei replicates. Since a single horizontal line could be drawn through the shaded region in each panel, there is no evidence that Johrei rates differ from those for controls or that the rates change over the duration of the experiment. The lowess command in STATA version 9 was used to perform the smoothing.

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