Therapeutic Touch Affects DNA Synthesis and Mineralization of Human Osteoblasts in Culture

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ABSTRACT: Complementary and alternative medicine (CAM) techniques are commonly used in hospitals and private medical facilities; however, the effectiveness of many of these practices has not been thoroughly studied in a scientific manner. Developed by Dr. Dolores Krieger and Dora Kunz, Therapeutic Touch is one of these CAM practices and is a highly disciplined five-step process by which a practitioner can generate energy through their hands to promote healing. There are numerous clinical studies on the effects of TT but few in vitro studies. Our purpose was to determine if Therapeutic Touch had any effect on osteoblast proliferation, differentiation, and mineralization in vitro. TT was performed twice a week for 10 min each on human osteoblasts (HOBs) and on an osteosarcoma-derived cell line, SaOs-2. No significant differences were found in DNA synthesis, assayed by [3H]-thymidine incorporation at 1 or 2 weeks for SaOs-2 or 1 week for HOBs. However, after four TT treatments in 2 weeks, TT significantly increased HOB DNA synthesis compared to controls. Immunocytochemistry for Proliferating Cell Nuclear Antigen (PCNA) confirmed these data. At 2 weeks in differentiation medium, TT significantly increased mineralization in HOBs (p = 0.03) increased HOB DNA synthesis compared to controls. Immunocytochemistry for Proliferating Cell Nuclear Antigen (PCNA) confirmed these data. At 2 weeks in differentiation medium, TT significantly increased mineralization in HOBs (p = 0.016) and decreased mineralization in SaOs-2 (p = 0.0007), compared to controls. Additionally, Northern blot analysis indicated a TT-induced increase in mRNA expression for Type I collagen, bone sialoprotein, and alkaline phosphatase in HOBS and a decrease of these bone markers in SaOs-2 cells. In conclusion, Therapeutic Touch appears to increase human osteoblast DNA synthesis, differentiation and mineralization, and decrease differentiation and mineralization in a human osteosarcoma-derived cell line. © 2008 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 26:1541–1546, 2008

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Complementary and alternative medicine (CAM) techniques are commonly used in hospitals and private medical facilities, and 36% of Americans use some form of CAM.1 However, the underlying mechanisms of action and effectiveness of many of these practices are unknown. Among the type of CAM are numerous energy therapies such as Qi-gong, Reiki, Healing Touch, Johrei, etc., which are derived from ancient healing practices in numerous countries. One of these energy medicine techniques is Therapeutic Touch (TT), which is a five-step process by which a practitioner focuses positive intentions on a subject and uses their hands, 4–10 inches away without touching, to facilitate self-healing in the patient. In the 1970s in the United States, Dr. Dolores Krieger and Dora Kunz pioneered and developed modern-day TT.2,3 TT was chosen for our studies because licensure requires a rigorous program, and the practice of TT is highly structured and disciplined, which would be more conducive for a scientific study to investigate its efficacy.

Clinically, TT has been shown to be effective in relieving tension headache pain in patients evaluated by the McGill-Melzack Pain Questionnaire.4 TT also significantly improved hand function for noninstitutionalized elderly adults suffering from arthritis.5 Another clinical study suggested that TT significantly reduced neurological complications during bone marrow transplant.6 In burn patients, TT may decrease pain perception, reduce anxiety, and enhance immune function.7 In several review articles and meta-analyses, clinical studies involving TT appeared to demonstrate clinical effects, but many studies, when evaluated as a whole, were considered to have weak experimental design and methodological limitations, such as questions about the appropriateness of the control group, the possible involvement of psychosocial factors, too small a sample size, etc.8–11 Therefore, it can be concluded that there is a need for a large standard, randomized controlled trial of TT’s efficacy. Few in vitro studies have been performed with TT; however, Radin and Yount12 demonstrated that TT increased the number of colonies of human astrocytes, if TT was applied repeatedly, but not from a single application of TT.

Our intent was to investigate the possible in vitro effects of TT on bone cells with controlled experiments that could be rigorously analyzed with statistics. In vitro osteoblast systems are excellent tools to study the effect of specific factors on the growth and differentiation of cells in a controlled environment with little to no contamination with other cell types or tissue factors. The human osteoblasts cultures are more than 95% osteoblasts as shown by immunocytochemistry for alkaline phosphatase,13 and this is due to the method by which they are isolated, which precludes other cell types. In brief, all soft tissue is removed from the bone chips obtained from patients, and the bone chips are placed in culture. Outgrowth of cells occurs only after 2 weeks in a defined medium that inhibits the survival of fibroblasts, blood cells, and other contaminating cells. The use of HOB cell cultures is also a preferred method for studying osteoblasts because these are normal human cells and are not an immortalized or transformed cell line from an animal. To determine if there is a biological response, we
chose to perform TT on cell cultures, instead of on patients, due to mind—body responses in a clinical study, which would further complicate the interpretation of the results. TT was performed on human osteoblasts isolated from patients undergoing orthopaedic procedures, and these results were compared to the effect of TT on a human osteosarcoma cell line (SaOs-2) that we have previously shown to differentiate and mineralize in vitro.\textsuperscript{13,14} The SaOs-2 cells were chosen due to their ability to form rapidly substantial amounts of calcified matrix in vitro.

**MATERIALS AND METHODS**

**Cell Culture**

Human bone fragments, discarded during the course of orthopedic surgery, were obtained from healthy patients undergoing foot surgery. All of the patients were in good health and were below the age of 50, because we have previously found decreased mRNA levels of bone matrix proteins and mineralization in HOBs from elderly female patients compared to males and other ages of patients.\textsuperscript{19} In addition, only the HOBs from one patient were used for all the groups and assays in one experiment, whereas a second experiment utilized HOBs from a different patient, etc. The University of Connecticut Health Center Institutional Committee on Human Research approved the use of discarded bone fragments. The bone fragments were immediately minced and then cultured in DMEM/F12 Ham (Sigma, St. Louis, MO) with 15% fetal bovine serum (FBS) and 100 units/mL penicillin G and 100 μg/mL of streptomycin sulfate.\textsuperscript{15} Cells were allowed to grow out of the bone chips for 2–3 weeks. Then the chips were removed and the cultures were allowed to become almost confluent. Human osteoblast-like cells (SaOs-2), derived from an osteosarcoma and obtained from American Type Culture Collection (Rockville, MD), were cultured in RPMI medium with 8% FBS and 100 μg/mL of streptomycin sulfate.\textsuperscript{16,17} When almost confluent, HOBs and SaOs-2 cells were harvested with 0.01% trypsin and were replated at 10,000 cells/cm$^2$ in six-well dishes. For mineralization studies, the medium was changed after 3–4 days in culture to α-MEM supplemented with FBS and antibiotics, and 50 μg/mL of ascorbate was added daily.

**Therapeutic Touch**

Three TT practitioners participated in this study. They were Registered Nurses, who were trained in TT and had more than 5 years each of TT practice on patients. They had also passed a TT screening test administered at the University of Connecticut Health Center prior to the start of this study. Neither the senior investigator nor the technicians who performed the assays were TT practitioners, and they had little knowledge of energy medicine. The practice of TT involves (1) “centering,” in which the practitioner focuses his/her positive intentions towards the subject; (2) “assessment” of the subject without touching the plate and remaining at least 4 inches away; (3) “unruffling” by using sweeping motions; (4) “treatment” involving the directing of positive intentions through the hands; and (5) reassessment. TT is performed by holding the hands 4–10 inches away from the area of interest and was adapted to our in vitro study. Treatments were 10 min in length and were administered twice a week with at least 1 day in between treatments. This protocol was determined by the TT practitioners in conjunction with the authors. Control (untreated) and “treatment” tissue cultures plates were clamped in one of two ring stands on a bench top, and were approximately 15 inches from the benchtop so that the practitioner hands could reach all sides without touching. Control and treated plates were positioned at either end of an L-shaped laboratory. Treatment was alternately performed on either end of the room with the treated plates receiving treatment twice a week and the untreated plates remaining clamped for the same time period while treatment was being performed on the other end of the room. Then the tissue culture plates were returned to the same incubator. Positioning of the plates in the incubator was random, and a technician with no knowledge of TT, set up the plates and returned the plates to the incubator. To consider the possibility that the effect of TT may originate from a physical or common mechanism shared by all humans, selected experiments were performed with a placebo control (an individual with no knowledge of TT who stood in front of identically prepared cell culture plates and used similar hand movements 4–10 inches away). However, the placebo individual counted backwards from 1000 for 10 min to distract and prevent any intentions.

**DNA Synthesis**

During the last 4 h of culture, [\textsuperscript{3}H] thymidine (5 μCi/mL) was added. The cell layers were extracted twice for 5 min with 10% trichloroacetic acid (TCA) and lysed in 0.5 N NaOH. Liquid scintillation counting was performed to measure radioactivity in the lysates (Packard Instrument Co., Downers Grove, IL). Five experiments with six replicates were assayed.

To confirm the effect of TT on proliferation determined by thymidine incorporation, an immunocytochemical staining method was used in three experiments with three replicates. Cells were fixed in 70% ethanol overnight. Immunocytochemistry for Proliferating Cell Nuclear Antigen (PCNA) was performed according to Zymed’s PCNA Kit (Zymed, South San Francisco, CA).

All assays were performed in a “blinded” manner without knowledge of the groups. The groups were identified after the data were complied.

**Mineralization**

At 2 and 4 weeks of culture, cells were extracted twice for 30 min with 5% TCA. Calcium content in the cell extract was measured colorimetrically using a calcium kit (Sigma, St. Louis, MO). Four HOB and six SaOs-2 experiments were performed with six replicates/experiment. An experiment with HOBs was also performed with 2-, 4-, and 6-week time points.

In two experiments, mineralization was normalized to DNA content by fluorometric analysis. The cells were disrupted with 0.01% SDS. Cell lysates were transferred to a 96-well plate, and 10 μg/mL H33258 was added to the cell lysate. DNA content was measured at 360-nm excitation and 460-nm emission by a microplate reader (Bio-TEK, Inc., Winooski, VT).

**Northern Blot Assay**

After 2 weeks of culture, total RNA was extracted with Qiagen’s RNeasy Protect Mini Kit (Qiagen Inc., Valencia, CA). RNA was separated by electrophoresis in 1% agarose containing 2.2-M formaldehyde and was transferred to a nylon membrane via PosiBlot (Stratogene, La Jolla, CA). The RNA was immobilized using Stratalinker (Stratogene),
prehybridized, and then hybridized at 42°C. The 21(1) procollagen (COL) and actin cDNA probes were kindly provided by Dr. Barbara Kream,18 the 1.8-kb bone sialoprotein (BSP) cDNA probe was generously obtained from Dr. Marion Young,19 and the 2.4-kb alkaline phosphatase (ALP) cDNA probe was kindly provided by Dr. Robert Majeska.20 The inserts were labeled with [32P] dGTP using random primer nucleotides. The filter was washed and exposed to Fuji RX film at −20°C for at least 24 h. Band densities were measured by SigmaScan software (Jandel Corporation, San Rafael, CA) and normalized to the 18S RNA. Three experiments were performed for each cell type.

### Statistics

Generally, most replications of an experiment involved three sets of six-well plates. DNA synthesis, mineralization, and a Northern blots were performed in one experiment. HOBs and SaOs-2 cells were plated in different dishes, treated separately, but in the same TT session. Both HOBs and SaOs-2 were analyzed from one experiment in a blinded, nonbiased manner with coding and with the same biochemical assay at the same time.

Data analysis focused on comparing the distribution of levels of proliferation and mineralization across study conditions, for example, “therapeutic touch versus control” or “therapeutic touch versus control versus placebo.” All comparisons used “exact” nonparametric statistical tests. Nonparametric tests were selected because study measures typically did not follow normal distributions and sometimes exhibited clear evidence of heterogeneity of variance between groups. Exact versions of the tests were performed to avoid reliance on “large-sample” approximations in the calculation of p-values.

For two-group comparisons (therapeutic touch versus control) that involved combination of observations across replicated experiments, the stratified Wilcoxon rank sum test proposed by Lehmann was used.21 This technique involves ranking observations within experiments to account for “block effects” that may vary from one experiment to the next. The significance level for these comparisons was set at 5%. For three-group comparisons (therapeutic touch vs. control versus placebo) that involved combination of observations across replicated experiments, the Lehmann technique was applied to each of the three possible pairwise comparisons of groups, and the Bonferroni method was used to account for potential inflation of the Type I error through multiple testing.22 Thus, in these circumstances, a 1.67% significance level was applied to each pairwise comparison to keep the overall probability of a Type I error in any comparison at or below 5%. The Bonferroni method is known to be “conservative” in that the “effective” probability of any Type I error is often less than the nominal, desired level of 5%.22

In one case, a three-group comparison was conducted that did not involve combination of data from different experiments. In this circumstance, the Kruskal-Wallis method provided the basis for initial testing. When that test provided a statistically significant result, the Wilcoxon rank sum test was used in conjunction with the Bonferroni correction to evaluate all pairwise comparisons. In another case, two groups (therapeutic touch vs. control) were compared at three time points (2, 4, and 6 weeks) using data from a single experiment. The difference in distribution between groups was assessed at each individual time point using the Wilcoxon test and the Bonferroni correction was applied to account for multiple testing across time points.

All statistical analyses were performed using StatXact software (version 4, Cytel Software Corporation, Cambridge, MA).

### RESULTS AND DISCUSSION

DNA synthesis was determined by [3H]-thymidine incorporation and was corroborated by immunocytochemical staining for proliferating cell nuclear antigen (PCNA) in a blinded nonbiased manner. In osteoblast cultures, proliferation occurs in the first 2 weeks and results in near confluence by 2 weeks. After 2 weeks, cells start to multilayer into nodules, which are the sites for mineralization.23 Therefore, DNA synthesis in osteoblast cultures was assessed at 1 and 2 week time points. One week of TT treatment was unable to produce a significant effect on [3H]-thymidine incorporation in SaOs-2 or HOB cultures. In the first week of treatment, a range of doses from one to five times of TT treatment was unable to elicit a significant effect in HOBs or SaOs-2 cells compared to untreated controls. However, other cell types such as fibroblasts and tenocytes demonstrate significant TT effects in the first week of treatment.24 TT significantly increased HOB tritiated thymidine incorporation by 316% after 2 weeks of treatment compared to controls (Fig. 1A) (p = 0.03, determined by the stratified Wilcoxon rank sum test referenced in Materials and Methods, N = 3 experiments). PCNA staining confirmed the [3H]-thymidine data and demonstrated that 2 weeks of TT treatment produced an increase in PCNA-stained HOBs compared to untreated HOB cultures (Fig. 1B). The majority of PCNA-stained cells were found at the periphery of developing nodules. No effect was found with SaOs-2 cultures at 2 weeks (p = 0.24, N = 5 experiments). The PCNA cells were quantified in eight random light microscopy fields of HOBs, and 25% ± 2 cells in the control group and 39% ± 3 cells in the TT group at 2 weeks stained for PCNA. The difference between treated and untreated HOBs was significant (p = 0.01). In additional studies with SaOs-2 cells, other TT doses were unable to produce any significant effects.

Further investigation of the expression of PCNA and [3H]-thymidine incorporation is needed in the future to determine if these assays are measuring proliferation alone or if unscheduled DNA synthesis due to DNA repair or apoptosis-related events are occurring.

A placebo control group was added to the [3H]-thymidine experiments with HOBs to determine if training and experience of the practitioner play any role in the outcome. Figure 1C demonstrated that TT stimulated HOB DNA synthesis after 2 weeks (p = 0.04) but the placebo individual did not stimulate DNA synthesis. In the post hoc pairwise comparisons that followed this statistically significant finding, the Bonferroni adjustment required application of a significance level equal to 0.0167. None of the comparisons fell below this more rigorous threshold (control vs. TT, p = 0.095; control vs. placebo, p = 0.017; TT vs. placebo, p = 0.43).

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suggesting that the experiment was underpowered to support the conservative Bonferroni approach. However, the results are suggestive of the possibility that the training and intention of an experienced practitioner may be required to elicit an effect.

The effect of TT on mineralization was determined by a quantitative biochemical method with all samples coded so that the technician has no knowledge of the treatment groups. After 2 weeks of TT treatment, the treated group (TT) showed an increase in staining when compared to the untreated group (C). Brown-stained cells (arrows) illustrate more cells expressing PCNA in the TT group compared to the control group. Bar = 10 μm.

The same experiment by the same practitioners, and the effects were opposite for HOBs and SaOs-2 cells, suggesting that the experiment was underpowered to support the conservative Bonferroni approach. However, the results are suggestive of the possibility that the training and intention of an experienced practitioner may be required to elicit an effect.
cell line. A time-course experiment with only HOBs demonstrated mineralization at 2, 4, and 6 weeks of culture with and without TT (Fig. 2B). TT was able to increase mineralization compared to untreated even at 4 and 6 weeks of TT treatment. However, once again the study may have been underpowered to support use of the conservative, Bonferroni approach to performance of multiple, pairwise statistical tests. The p-values at 4 and 6 weeks were both equal to 0.029. Although these p-values fell below the nominal 0.05 cutoff for significance, they did not reach the more extreme threshold of 0.0167 required by use of the Bonferroni method. Two experiments were also performed with a placebo control SaOs cells as described in Materials and Methods, and the calcium content was normalized to DNA (Fig. 2C). Control compared to the TT groups obtained a p-value of 0.019, control compared to placebo reached a p-value of 0.75, and TT compared to placebo was significantly different, after Bonferroni correction, at p = 0.003.

Because bone formation occurs by the sequential synthesis and secretion of extracellular matrix proteins to form osteoid which subsequently mineralizes, Northern blot analyses of message levels for the bone matrix proteins; alkaline phosphatase (AP), bone sialoprotein (BSP), and Type I collagen, were performed in three separate experiments. At 2 weeks of TT treatment on HOB cultures, BSP, AP, and Type I collagen message levels increased 44, 81, and 39%, respectively, compared to untreated cells (Fig. 3A). These bands were normalized to actin, and percent relative density is demonstrated in the graph. After a 2-week application of TT to SaOs-2 cultures, BSP, AP, and Type I collagen mRNAs decreased 71, 88, and 59%, respectively, compared to untreated controls (Fig. 3B). These bands were normalized to actin. Northern blot analysis of message levels for bone matrix proteins from HOB and SaOs-2 cells correlated with the calcium content data, and demonstrated that TT significantly affected mineralization in vitro. In both cell types, Northern blot analyses demonstrated similar changes in three experiments.

Although there is no knowledge to date on what type of energy may be emanating from a TT practitioner’s hands, it is conjectured that TT may affect biological changes through altering biomagnetic fields. All living organisms reveal a dynamic biomagnetic field that is indicative of both natural biological changes and abnormal biological states. Several studies reveal large pulsed biomagnetic fields emanating from the hands of practitioners in a variety of modalities including TT, Qi-gong, yoga, meditation, and martial arts. A shift in energy emission by practitioners performing Therapeutic Touch was measured in a superconducting quantum interference device (SQUID) magnetometer, and biomagnetic fields have been recorded at a frequency from 8 to 10 Hz from practitioners’ hands. Although the type of energy field, if any, that is induced by TT is unknown, electromagnetic fields, in particular, pulsed electromagnetic fields (PEMFs), have been shown significantly to stimulate bone formation. Low-

power, low-frequency PEMF (approximately 15 Hz), designed to induce voltages similar to those produced during normal mechanical deformation in connective tissue, have been shown to be most effective in eliciting osteogenesis and bone remodeling in a disuse model for osteopenia in adult turkeys, in the metacarpals of horses, and in a rabbit tibial osteotomy for the study of fracture healing. Lohmann et al. demonstrated that PEMFs decreased proliferation and increased differentiation in the osteoblast-like cell line, MG63.

Our findings reveal that TT can elicit biological effects in vitro, in particular in an established mineralizing culture system. The mechanism for these effects is yet to be elucidated, but the results provide a basis to study further the existence of a human biofield that may have some biological effects.

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