Tai Chi Improves Oxidative Stress Response and DNA Damage/Repair in Young Sedentary Females

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Abstract. [Purpose] This study was to examine the effects of 12 weeks of Tai Chi (TC) exercise on antioxidant capacity, and DNA damage/repair in young females who did not perform regular physical exercise. [Subjects and Methods] Ten female students from a Chinese university voluntarily participated in this program. All of them practiced the 24-form simplified Tai Chi, 5 times weekly, for 12 weeks. Plasma levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), glutathione (GSH), hydroxyl radical inhibiting capacity (OH·-IC), 8-hydroxy-2'-deoxyguanosine (8-OHdG), and 8-oxoguanine DNA glycosylase (OGG1) were measured at 0, 8, and 12 weeks. Heart rate (HR) was monitored during the last set of the training session at 4, 8, and 12 weeks. [Results] Plasma SOD and OH·-IC levels were increased at 8 and 12 weeks compared to the baseline (0 weeks). Gpx and GSH levels did not change significantly throughout the study period. The plasma MDA level was decreased significantly at 8 weeks but not at 12 weeks compared to the baseline value. While the plasma 8-OHdG level did not change throughout the study period, the plasma OGG1 level was significantly increased at 8 and 12 weeks compared to the baseline value. [Conclusion] TC practice for 12 weeks efficiently improved the oxidative stress response in young females who did not perform regular physical exercise. The TC exercise also increased the DNA repairing capacity.

Key words: Tai Chi, Oxidative stress, DNA damage

INTRODUCTION

Oxidative stress refers to an imbalance between free radical [especially reactive oxygen species (ROS)] generation and antioxidant defense3). The antioxidant system in the human body includes enzymatic [e.g. superoxide dismutase (SOD) and glutathione peroxidase (GPx)] and non-enzymatic antioxidants [e.g. glutathione (GSH)] and it plays an important role in the prevention of oxidative stress. Oxidative stress is involved in the pathogenesis of hypertension, atherosclerosis, diabetes, osteoporosis, and cancer2). It is also known to cause and accelerate the aging process3). ROS initiates lipid peroxidation through an attack on polyunsaturated fatty acids, and generates products such as MDA (malondialdehyde)9). ROS also causes oxidative damage to proteins and DNAs6). Among these damages, oxidative DNA damage is the most detrimental one to human health because of its role in the pathogenesis of the various diseases mentioned above. ROS, especially hydroxyl radical (OH•), can cause DNA base changes, strand breaks, damage to tumor-suppressor genes, and enhanced expression of proto-oncogenes5). In addition to the increase of ROS, the decrease of DNA repair capacity is the other reason for the accumulation of oxidative DNA damage in the human body6).

Physical exercise is an important factor affecting oxidative stress and oxidative DNA damage, since a sharp increase in oxygen consumption during exercise results in an increase in ROS generation. However, the effect of exercise-induced oxidative damage is variable depending on several factors such as type, mode, duration, and intensity of exercise7). A single bout of exercise induces oxidative stress and DNA damage, whereas regular moderate exercise intensity decreases them8). Under the concept of hormesis, low-to-moderate ROS generation induced by regular moderate intensity exercise is beneficial, since it would up-regulate key antioxidant enzymes9). A previous study demonstrated that long-term high-intensity exercise (75% VO2 max) increased 8-hydroxy-2'-deoxyguanosine (8-OHdG), while regular exercise with moderate-intensity (50% VO2 max) tended to decrease 8-OHdG10). The activity of 8-oxoguanine DNA...
glycosylase (OGG1), a crucial enzyme for the repair of 8-oxoguanine lesions, is up-regulated after regular exercise in human and animals\(^1\).

Tai Chi (TC), a traditional Chinese exercise, is classified as a moderate intensity exercise, and its intensity is basically similar among different age and gender groups\(^2\). Previous studies have reported the numerous tangible benefits of TC practice, such as improvement of balance and prevention of falls, enhancement of flexibility and strength, as well as amelioration of cardio-respiratory and immune function impairments. Studies of the effects of TC exercise on oxidative stress and DNA damage are limited. TC exercise reduced the degree of oxidative stress in middle-aged women\(^3\), older adults\(^4\), and obese patients with type 2 diabetes\(^5\), and long-term TC practice reduced the degree of DNA damage, measured by the comet assay, in middle-aged\(^6\) and older\(^7\) adults.

However, the effects of TC intervention on oxidative stress and DNA damage have not been investigated in young females who seldom participate in physical exercise. The efficacy of TC exercise on DNA base damage and its repair also remains unclear. Young females are less conscious about physical exercise compared to age-matched males\(^8\). Although TC exercise is suitable for any population\(^9\), only middle-aged and elderly age groups frequently practice TC for health purposes. TC is becoming popular among Chinese college students since it has been designated as a compulsory sport in the Physical Education curriculum of most colleges. Therefore, the objective of this pilot study was to determine the effects of TC intervention on oxidative stress, DNA damage and repair in young females who did not participate in regular physical exercise.

**SUBJECTS AND METHODS**

Ten sedentary female students from Gannan Normal University, China, voluntarily participated in this program. They completed inclusion and lifestyle questionnaires. Exclusion criteria were as follows: having experience of TC exercise; having performed physical exercise for more than 1 hr per week in the past 3 months; smoker or regular alcohol drinker; consuming supplements or medical products with antioxidant properties; having a history of cardiovascular and respiratory diseases; having knee joint pain; having a history of lower-extremity fracture within the past 1 year.

The subjects gave their informed consent to participate in this study. They were asked to keep their usual lifestyle including dietary behavior and daily activities, and medication (if any) throughout the study period. At the time of recruitment, their demographic characteristics were as follows: age, 20.40±0.70 yrs old; height, 155.48±6.15 cm; weight, 47.13±6.11 kg; body mass index (BMI), 19.42±1.54 kg/m\(^2\); and resting heart rate, 79.10±12.65 beats/minute.

All of the participants completed the 12-week TC training program. According to this protocol, they practiced the 24-form simplified TC, 5 times (sessions) per week (Monday to Friday), on campus. The first 2 weeks involved familiarization with TC through teaching and learning. In the following 10 weeks, the participants practiced 5 rounds of TC (1 round refers to practice from the 1st movement to the 24th movement of this TC style) accompanied by a classic music for this TC style in each session. An experienced TC instructor led all participants in practice and taught them necessary movement corrections in the intervals between rounds. Thus, each session lasted about 60 min including a 10-minute warm-up for stretching and a 5-minute cool-down for relaxation.

Heart rate (HR) during TC exercise was monitored in the last session of the 5 rounds of the 24-form simplified TC in the last 4th, 8th, and 12th weeks. The exercise duration of five rounds of TC practice lasted for around 30 minutes. HR parameters such as average HR, maximum HR, and minimum HR were recorded using a Polar watch (Suunto t6d Running pack, Finland) and the telemetry HR team system (Suunto team manager, Finland).

Blood samples were collected at baseline, 8, and 12 weeks. In each session of blood collection, 3 mL of whole blood was taken after overnight fasting between 7:30–8:30 a.m. from a forearm vein and placed into a heparinized tube. Plasma was separated by centrifugation at 3,000 rpm for 5 min and kept frozen at −80°C until assayed for 8-OHdG, OGG1, SOD, GPx, MDA, GSH, and hydroxyl radical inhibiting capacity (OH·-IC).

SOD was measured by the hydroxylamine assay using a spectrophotometer (UNICO7200, USA) at 550 nm. GPx was detected by the rate method using a spectrophotometer (UNICO7200) at 412 nm. MDA was assessed by the thiobarbituric acid (TBA) assay using a spectrophotometer (UNICO7200) at 532 nm. GSH was analyzed by colorimetry using a spectrophotometer (UNICO7200) at 420 nm. Hydroxyl radical inhibiting capacity was determined by the Griess colorimetric method using a spectrophotometer (UNICO7200) at 550 nm. The kits for SOD, GPx, MDA, GSH, and OH·-IC measurements were all purchased from Nanjing Jiancheng Bioengineering Institute, China.

Plasma 8-OHdG and OGG1 levels were measured using commercial ELISA (enzyme-linked immunosorbent assay) kits according to the manufacturer’s instructions (Santa Cruz Biotechnology, Inc. Dallas, Texas, USA). Briefly, 40 µL of plasma were added to the sample wells. Then, either 10 µL of anti-8-OHdG or anti-OGG1 antibody in combination with 50 µL of streptavidin-biotin-horseradish peroxidase were added to the sample wells in turn. To the standard sample wells, 50 µL of 5 different concentrations of standard samples and 50 µL of streptavidin-biotin-horseradish peroxidase were added. The microtiter plate was then incubated for 1 hour at 37 °C. Then, the plate was washed with wash buffer 5 times. During the procedure of washing, before discarding the buffer, each well was filled with wash buffer for 30 seconds. Color reactions were then conducted for 10 min in the dark at 37 °C after 50 µL of chromogenic agent A and 50 µL of chromogenic agent B were added in turn to each well. Finally, 50 µL of stop solution was added to terminate the reaction and the blue color changed to yellow. Products were then detected with the medical Elisa Analyzer (DG5033A, Huadong Electronics Group Co., Ltd., Shanghai, China) at 450 nm within 15 min, and the concentrations of 8-OHdG and OGG1 in plasma were read.
from standard curves.

All data were expressed as the mean ± SD. The differences of plasma and HR monitoring parameters among different time points were determined by repeated measures analysis of variance (ANOVA). Post hoc pair-wise comparisons (LSD) were performed to test the differences when significance was shown. The correlation between plasma 8-OHdG and OGG1 levels at the same time point was determined using Pearson’s correlation coefficient. The Statistical Package for Social Sciences (SPSS) version 17.0 was used for the analysis and values of p<0.05 were considered significant.

RESULTS

When the participants were performing 5 rounds of TC, the average HR and maximum HR tended to increase at 4, 8, and 12 weeks, but they did not show significant differences (Table 1). In contrast, the minimum HR tended to decrease during this study period, although the decreases were not statistically significant (p>0.05) (Table 1). The SOD level was significantly increased (p<0.05) at 8 and 12 weeks of TC exercise compared to the baseline value (Table 2). OH·-IC was also significantly increased at 8 and 12 weeks. Conversely, the plasma MDA level was significantly decreased at 8 weeks but not at 12 weeks compared to the baseline (Table 2). However, GPx and GSH levels did not alter significantly during the study period.

Although the increases were not statistically significant, the plasma 8-OHdG values in this study showed increases at 8 and 12 weeks compared to the baseline value (Table 3). In contrast, the plasma OGG1 level was significantly increased at 8 and 12 weeks compared to the baseline value (Table 3). Positive correlations between plasma 8-OHdG and OGG1 were found at baseline (p=0.003) and 8 weeks (p=0.008), but no statistically significant correlation (p=0.103) was found at 12 weeks (Table 3).

Table 1. Comparison of HR among the different time points

| Variables     | 4 weeks   | 8 weeks   | 12 weeks  |
|---------------|-----------|-----------|-----------|
| HRaverage (bmp) | 108.3±10.5 | 110.00±10.7 | 113.9±18.8 |
| HRmax (bmp)    | 124.2±12.4 | 126.9±13.4 | 132.9±22.5 |
| HRmin (bmp)    | 88.3±10.9  | 87.7±8.3   | 84.8±10.4  |

HRaverage = mean heart rate, HRmin = minimum heart rate, HRmax = maximum heart rate, bmp = beats per minute

Table 2. Effects of Tai Chi exercise on oxidative stress markers

| Variables     | baseline   | 8 weeks    | 12 weeks   |
|---------------|------------|------------|------------|
| SOD (U/ml)    | 42.76±3.05 | 49.81±5.01*| 49.05±4.73*|
| Gpx (U/ml)    | 130.30±22.88 | 133.53±18.77 | 141.60±28.55 |
| MDA (nmol/ml) | 2.77±0.41  | 2.12±0.41* | 2.97±0.54  |
| GSH (mgGSH/L) | 11.92±5.07 | 10.48±3.08  | 9.19±4.56  |
| OH·-IC (U/ml) | 351.97±59.02 | 406.19±34.76*| 408.49±49.86*|

*p<0.05 compared to baseline, SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malondialdehyde; GSH: glutathione; OH·-IC: hydroxyl radical inhibiting capacity

Table 3. Comparison of plasma 8-OHdG and OGG1, and their relationships at different time points

| Variables     | baseline   | 8 weeks    | 12 weeks   |
|---------------|------------|------------|------------|
| 8-OHdG (ng/ml) | 9.45±2.54  | 10.93±2.51 | 10.26±2.34 |
| OGG1 (ng/ml)  | 6.91±2.05  | 8.17±1.54* | 8.49±1.62* |
| R (p value)   | 0.842 (0.003) | 0.78 (0.008) | 0.545 (0.103) |

*p<0.05 compared to baseline, 8-OHdG: 8-hydroxy-2’-deoxyguanosine; OGG1: 8-oxoguanine DNA glycosylase; R: correlation coefficients of plasma 8-OHdG and OGG1 at the same time point

DISCUSSION

Several empirical studies using different parameters, including heart rate (HR), oxygen consumption (VO2), and energy expenditure, have shown that TC is an aerobic exercise of moderate intensity12). However, in those previous studies, the intensity of TC was measured only when participants practiced one round of TC, from the first movement to the last one of the specific TC style. It is intuitive that several factors such as the individual variances in age, gender, proficiency levels of TC, and also TC style would affect the intensity. In the present study, we monitored the
participants’ HR when they practiced 5 rounds of the 24-form simplified TC at 4, 8, and 12 weeks. The results of HR monitoring show that the average HR during TC practice corresponded to 54.3%, 55.1%, and 57.1% of the age-predicted maximum heart rate (=220-age) at 4, 8, and 12 weeks, respectively. These results indicate that the intensity of TC practice of the present protocol was moderate for the young female students.

Although exercise has numerous benefits for human health, it is also often claimed to induce oxidative stress since exercise increases ROS generation. About 2–5% or more of inhaled O₂ is converted into various ROS byproducts. ROS generated during exercise is, however, not always harmful because moderate and gradual exercise helps people to acquire antioxidant defenses.

The antioxidant defense system in the human body includes enzymes, such as SOD and GPx, and non-enzymatic antioxidants such as GSH. In the enzymatic system, SOD is the first defense against oxidative stress and is the major defense against O₂⁻, whereas GPx is responsible for scavenging H₂O₂ and other organic peroxides. GPx requires GSH as a substrate for peroxide decomposition. In the present study, we observed a significant increase in plasma SOD and OH⁻IC levels after 8 weeks of TC exercise indicating that 8 weeks of TC exercise following our protocol is sufficient enough to improve the scavenging capacities for O₂⁻ and OH⁻. In this study, the plasma MDA level decreased at 8 weeks. Thus, TC exercise can help to reduce the lipid peroxidation products attacked by ROS. In the present study, although the increase was not statistically significant, GPx tended to increase with time of TC practice. Since GPx is efficiently induced by high ROS concentrations, our protocol would have generated low concentrations of ROS. Our results are in agreement with those of previous studies concerning the protective effects of TC exercise on oxidative stress in middle-aged and elderly subjects.

We propose there are two possible mechanisms of regular moderate-intensity exercise which reduce the markers of oxidative stress. First, based on the theory of hormesis, cumulative effects of repeated exercise bouts and exposure to ROS (especially OH⁻) attack at the C8 position of deoxyguanosine in DNA. The damaged DNA with 8-OHdG is formed by OH⁻ attack on normal guanine. An animal study also showed that 2 months of regular treadmill running significantly upregulated the activity of OGG1 in hepatocyte nuclei of old rats compared to a sedentary old group. Another animal study showed that exercise training increased OGG1 levels/activity in the nucleus of skeletal muscles and specific activity of OGG1 in mitochondrial compartments. Similarly, our present study demonstrated that the plasma OGG1 level significantly increased after TC practice for 8 and 12 weeks. Increase of this enzyme in plasma is likely to be an adaptive response to the increased oxidative damage induced by elevated ROS during TC exercise.

8-OHdG is formed by OH⁻ attack at the C8 position of deoxyguanosine in DNA. The damaged DNA with 8-OHdG may contribute to point mutation during the subsequent replication. With normal body repair mechanisms, 8-OHdG can be cleaved by OGG1 through the way of base excision pathway and normal guanine is added to the site. A few studies have reported a correlation between 8-OHdG and OGG1. For example, Kondo et al. showed a positive association between 8-OHdG levels and OGG1 expression (r=0.702, p<0.05). In our study, significant positive correlations between plasma 8-OHdG and OGG1 were observed at baseline (r=0.824, p<0.01) and 8 weeks (r=0.78, p<0.01); however, no significant correlation was found at 12 weeks (r=0.545, p>0.05). These results suggest that the increase of OGG1 activity after 12 weeks of TC exercise exceeds the requirements for immediately excising all 8-OHdG in young females. The enhancement of OGG1 may increase the repair rate.

Based on the results of this pilot study, a 12-week TC exercise intervention is effective at increasing the activity of plasma SOD and OGG1 and decreasing plasma MDA concentration in young sedentary females. It suggests that TC exercise helps to alleviate the oxidative stress response and DNA damage in those who have serious levels of oxidative stress and DNA damage, such as older and sick people.

This study had several limitations. First, there was no control group. Second, the sample size was small. Third, the authors only told the participants to keep their daily dietary habits and did not ask them to record their dietary details, especially before each blood sampling. Thus, dietary variation might have affected the data of blood parameters. Nevertheless, this pilot study showed TC intervention can improve the response to oxidative stress and the DNA damage/repair process in female students. Further studies with a longer intervention duration, larger groups, and a control group are needed to confirm our present results. Moreover, the long-term effects of Tai Chi on oxidative stress response
and DNA/repair also need to be studied in the future.

ACKNOWLEDGEMENTS

This study was supported by the Back, Neck and Other Joint Pain Research Group, Khon Kaen University, Thailand. We also thank Professor Yukifumi Nawa for his helping with the modification of this manuscript.

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