Anti-Stress Effect of Oolong Tea in Women Loaded with Vigil

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The preventive effect of oolong tea on vigil stress was investigated in 55 Chinese women. The subjects received 4 servings of tea bag daily, the tea bags in each containing 2 g dry weight of oolong tea or barley tea, or else water, for a period of one week. The results of a questionnaire indicated that ingestion of oolong tea improved symptoms of stress such as stiffness of the shoulders, fatigue of the eyes and headaches, as well as ameliorated the stress-induced increase in the number of errors in calculation tasks compared with controls. In addition, plasma cortisol levels were significantly lower in the oolong tea group (17.84 ± 2.46 μg/dl) than in the barley tea group (21.33 ± 6.47 μg /dl) or water group (22.95 ± 6.98 μg/dl). Ingestion of oolong tea significantly alleviated the vigil stress-induced increase in plasma lipid peroxide levels, which may have been related to the stress-relieving effects of caffeine or antioxidant properties of polyphenols contained in the tea. These findings suggested that oolong tea has anti-stress effect, with no adverse effects on appetite or physical fitness.

Key words —— oolong tea, stress, cortisol, lipid peroxide

INTRODUCTION

Stress is involved in various diseases, and there are many stressors in our environment. There have been a number of recent studies about how the signs and symptoms of stress arise. A response to stress is transmitted to the organs through the autonomic nervous system and hormones.[1)] Stress directly affects the secretion of hormones,[2)] to suppress the immune system,[3)] and can cause acute organ dysfunction.[4)] For example, some stresses reduce the production of insulin in animals and impair glucose metabolism.[5)] When less glucose is used as energy, not only fatigue but also physiological disorders may arise. Recently, stress has been reported to have a marked effect on cortisol production.[6)] Although cortisol is indispensable for homeostasis including gluconeogenesis,[7)] an excess secretion of cortisol induced by stress brings about abnormalities in lipid and protein metabolism and can result in the development of life style-related diseases.[8)] In addition, it is also well known that various active oxygen, free radicals are formed by stress.[9)] Superoxide radicals are known to damage cell components causing aging and several serious diseases, such as cancer,[10)] and lipid peroxide to form.[11)] Lipid peroxide is considered injurious to immunocompetent cells involved in the inhibition of immune function.[12)] Therefore, it is very important to find effective scavengers of superoxide radicals. Early studies indicate that some tea catechins inhibit lipid oxidation and scavenge superoxide radicals.[13)] Serafini *et al.*[14)] found that the ingestion of tea inhibits lipid peroxide production in humans, and Lin et al. found the same effects in rats.[15)] Tea also reduces DNA damage caused by oxidative agents *in vitro*.[16)] It is possible to use plasma cortisol and lipid peroxide levels as an index for the anti-stress effects of oolong tea.

Oolong tea and green tea are the most popular beverages in Japan and China, while black tea is preferred in America and Europe.[17)] All types of tea are manufactured from the same plant species, *Camellia sinensis* L., which was first discovered in

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China where it has been used as a daily beverage known to have beneficial effects on health for thousands of years.[18] The various kinds of tea are produced through different processing methods. Oolong tea is semi-fermented, green tea is not fermented, and black tea is well fermented. In China, oolong tea traditionally has been considered to have anti-obesity and hypolipidemic effects and it has been thought that habitual ingestion is effective in enhancing metabolic rates and fat oxidation.[19]

However, less attention has been given to the influence of oolong tea on stress. Therefore, we examined the effects of oolong tea on vigil stress in 55 Chinese women. The study was approved by the ethical committee of the institute of Traditional Chinese Medicine of Fujian Province, according to the Declaration of Helsinki and written informed consent was obtained.

MATERIALS AND METHODS

Selection Criteria —— Fifty-five healthy Chinese women with a mean age of 20 ± 1 years participated in this study. They were all recruited from university students in the Fujian college of traditional chinese medicine. Medical and nutritional histories were obtained by use of a questionnaire. Smokers, drinkers, and habitual drug (liquid preparations, vitamins, tranquilizers, hypnotics *etc*.) users were excluded. The experiment director or test doctor explained the purpose of the experiment, test protocol and bioactivity of oolong tea prior to the experiment to all subjects. Then, informed consent to participate in this study was obtained.

Oolong Tea and Barley Tea —— Oolong tea was provided by Fujian Tea Import & Export Co., Ltd. (China) prepared in bags containing 2 g of tea per bag. The tea was brewed by adding 300 ml of boiling water to a glass container containing the tea bag. The tea was steeped for 5 min, and the bag was then removed. The concentrations of caffeine, gallic acid, flavanols, and other polyphenols in the oolong tea were analyzed by high-performance liquid chromatography (HPLC) with UV detection at 280 nm.[20] Analysis was performed with a Cosmosil 5PE-MS column (4.6 mm internal diameter × 150 mm; Nakarai Tesuque, Kyoto, Japan) at 40°C. Compounds were eluted (eluent A: 0.05% trifluoroacetic acid in water; eluent B: 0.05% trifluoroacetic acid in acetonitrile) at a flow rate of 2 ml/min using a gradient program (eluent B content: 10% for 5 min, 21% for 8 min, 90% for 1 min, and 90% for 6 min). The quantification of caffeine, gallic acid, and flavanols was determined using standard calibration curves for marketed compounds. Other polyphenols were quantified using a calibration curve that was derived from other polyphenols that had been isolated from tea by HPLC. The components of caffeine and tea polyphenols of oolong tea are shown in Table 1. Total caffeine and tea polyphenol consumption for subjects consuming 100 ml of the oolong tea were 23.51 and 99.32 mg, respectively.

Table 1. Components of Caffeine and Oolong Tea Polyphenols

Components	Oolong tea (mg/100 ml)
Gallic acid	2.19
Caffeine	23.51
Gallocatechine	6.68
Epigallocatechine	16.14
Catechine	1.65
Epicatechine	5.08
Epigallocatechine gallate	25.73
Allocatechine gallate	1.85
Epicatechine gallate	5.73
Catechine gallate	0.60
Polymerized	33.65
Total polyphenols	99.32

Data are mean amounts of oolong tea components consumed daily.

Barley tea was obtained from Suntory Ltd. (Osaka, Japan) prepared with 2 g per bag. The tea was extracted by placing a tea bag for 5 min in 300 ml of boiling water and used as tea samples in the present study. On the other hand, a previous report indicated barley tea has no caffeine, but contains catechol and very small quantities of gallic acid and gentisic acid.[21]

Experimental Schedule —— In the present study, the 55 subjects were divided randomly into the oolong tea ingestion group (21 subjects), the barley tea ingestion group (14 subjects) and the water control group (20 subjects). The protocol was explained to them and the intake of anti-stress agents, psychoactive drugs and anti-oxidants was prohibited throughout the test period. No further restrictions were placed on meals or daily life except for prohibition of intensive exercise. Oolong tea and a corresponding quantity of barley tea as a placebo control were ingested twice in the morning and twice in the afternoon, the subjects received 4 servings of tea bag daily, the tea bags in each containing 2 g dry weight of oolong tea or barley tea, a total of 8 g daily, for

1 week before the vigil stress. For the water treatment, subjects drank water but no tea.

On the final administering day, overnight workloads were given to the three groups of subject, and placed in their classroom. During the period of vigil stress, subjects were allowed to intake normal food and moderate amounts of drink, respectively, but were forbidden to smoke. The subjects were asked to perform a calculation test as a stress index every two hours, starting from 11:00 pm at 5 time points overnight. Various clinical manifestations during the experiment were observed and recorded. The questionnaire was applied after the test. Biochemical markers were determined before (17:00) and after vigil stress (7:00). A questionnaire was also applied to monitor the stress index after the vigil test (7:00).

Clinical Physiology Index —— The subjects performed a calculation test consisting of 40 calculation tasks per sheet, and were instructed to give as many answers as possible within one minute per sheet at two-hour intervals. The number of errors and the ratio of correct to incorrect answers were calculated as stress indexes, and the results taken as the percentage of questions answered right or wrong to the total number in the five tests. Data for headaches, stiffness of the shoulders (back and joint pain) and fatigue of the eyes were recorded up to the study period.

Measurement of Cortisol and Lipid Peroxide Levels in Plasma —— Heparinized blood samples were obtained in blood collecting tubes. Samples were centrifuged immediately after collection (1000 g for 15 min). The plasma was separated and stored in aliquots at –80°C until subsequent use. Plasma cortisol levels were determined by the method of Henderson,[22] and plasma lipid peroxide levels were determined by the thiobarbituric acid-reactive substances (TBARS) method of Yonaha *et al.*[23]

Standard of Safety and Test Discontinuance —— In the present study, safety was evaluated as follows: safe (no side effects); borderline (slight side effects, but safe to continue); and unsafe (side effects, ingestion will be discontinued). The study director or test doctor was instructed to report a case with serious side effects causally related to the test. The test director was charged with deciding whether to discontinue the test after consultation with the subject who had serious side effects related to the test.

Statistical Evaluation —— Statistical analyses were performed with Student's *t*-test. Differences were considered to be significant when the probability value was less than 0.05. The results were given as the mean ± S.D.

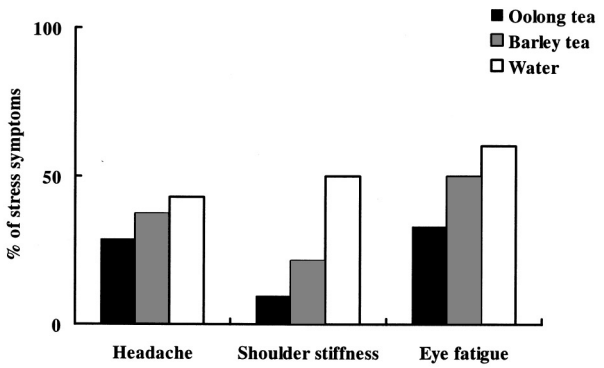

Fig. 1. Effects of Oolong Tea Ingestion on Various Stress Symptoms in Women Loaded with Vigil

Fifty-five healthy Chinese women participated in this study. Subjects received 4 servings of tea bag daily, the tea bags in each containing 2 g dry weight of oolong tea or barley tea, or else water only, for a period of one week. The clinical symptoms were recorded during the period of vigil stress. Each bar represents mean values of the score for stress symptoms in women loaded with vigil.

RESULTS

Effects of Oolong Tea Ingestion on Various Clinical Symptoms

The results of the questionnaire indicated that stress indexes such as headaches, stiffness of the shoulders and fatigue of the eyes increased during the vigil stress period (Fig. 1). These stress indexes were 42.8%, 50% and 60% in the water ingestion group, and 37.1%, 21.4% and 50% in the barley tea group, respectively. In the oolong tea group, these values were 28.5%, 9.5% and 32.8%, respectively. Oolong tea ingestion improved these stress indexes compared with the same dose of barley tea and water. On the other hand, ingestion of barley tea also slightly ameliorated vigil stress as compared to the water control.

Effects of Oolong Tea Ingestion on the Test Error Rate

We investigated the effects of vigil stress on the error rate in the calculation test, as a stress index during the vigil stress period. In the first test, the error rate was no significant difference among each group, however the oolong tea group decreased number of error rate from the next test to the final test. The results were shown as an average of error rates in the five tests (in Fig. 2). Thus, the water, barley

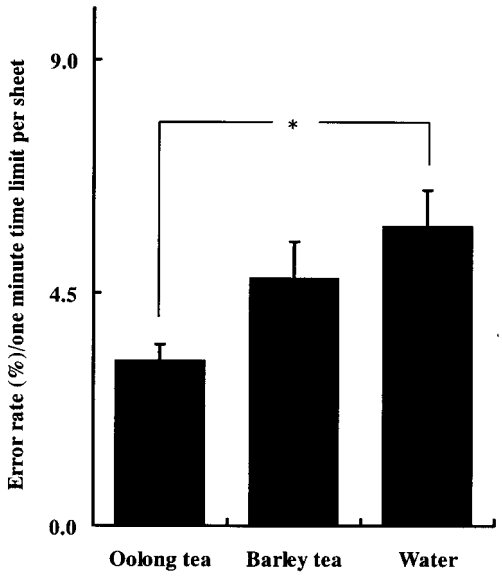

Fig. 2. Effects of Oolong Tea Ingestion on the Calculation Test in Women Loaded with Vigil

Fifty-five healthy Chinese women participated in this study. Subjects received 4 servings of tea bag daily, the tea bags in each containing 2 g dry weight of oolong tea or barley tea, or else water only, for a period of one week. Each bar represents the mean ± S.D. for the calculation test and a statistically significant difference from the control at $p < 0.001$ as determined with Student's *t*-test.

tea, and oolong tea ingestion groups were 5.84 ± 0.75, 4.82 ± 0.73, and 3.15 ± 0.33, respectively. Oolong tea ingestion alleviated the vigil stress ($p < 0.005$), compared with the same dose of barley tea or water.

Effects of Oolong Tea Ingestion on Plasma Cortisol Levels

We investigated the mean levels of plasma cortisol in normal daily life for the 55 subjects before the study. The anti-stress activity of the oolong tea was evaluated based on the plasma cortisol levels. Figure 3 shows that the normal plasma cortisol level was 7.8 ± 1.6 μg/dl in the afternoon (17:00), and 14.1 ± 1.6 μg/dl in the morning (7:00). Although the level increased after vigil stress in the oolong tea group (17.84 ± 2.46 μg/dl) in the morning (7:00), it was significantly lower than in the barley tea group (21.33 ± 6.47 μg/dl) and water group (22.95 ± 6.98 μg/dl). The ingestion of oolong tea significantly ($p < 0.05$) alleviated the vigil stress-induced increase in plasma cortisol levels as compared with barley tea or water (Fig. 4).

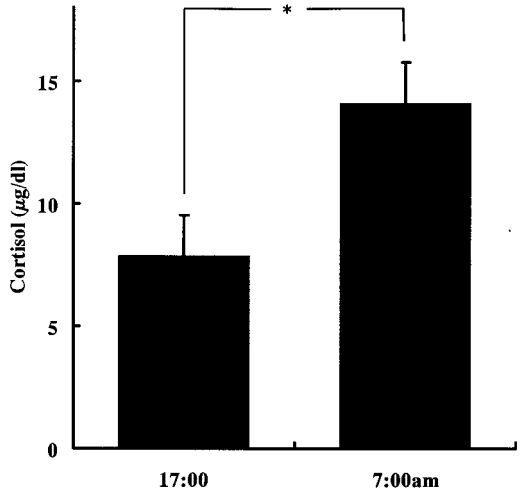

Fig. 3. Plasma Cortisol Levels in Normal life Obtained from 55 Healthy Chinese Women

Mean changes of plasma cortisol levels were calculated for afternoon (17:00) and morning (7:00) in normal life. Each bar represents the mean ± S.D. and a significant difference from the control at $p < 0.001$ as determined with Student's *t*-test.

Effects of Oolong Tea Ingestion on Plasma Lipid Peroxide Levels

Plasma lipid peroxides were measured at baseline levels in the 55 subjects before the study. However, in contrast to the plasma cortisol level, the plasma lipid peroxide level was lower in the morning (at 7:00 it was 3.18 ± 0.31 nmol/ml) than in the afternoon (at 17:00 it was 3.79 ± 0.53 nmol/ml) (Fig. 5). The results suggested that this low value may be related with sleep. As shown in Fig. 6, the plasma lipid peroxide level in the morning was increased by vigil stress load as compared with the levels in normal daily life. The value in the water ingestion group was 4.10 ± 2.10 nmol/ml, that in the barley tea ingestion group was 3.38 ± 1.39 nmol/ml, and that in the oolong tea ingestion group was 3.15 ± 0.33 nmol/ml. Compared with barley tea or water, oolong tea significantly alleviated the vigil stress-induced increase in plasma lipid peroxide levels ($p < 0.05$).

DISCUSSION

In the present study, we recruited 55 subjects and randomly allocated to them receive oolong tea, barley tea, or drinking water for 1 week before assigning a workload overnight. Our results show that oolong tea ingestion improved various symptoms of stress such as stiffness of the shoulders, fatigue of

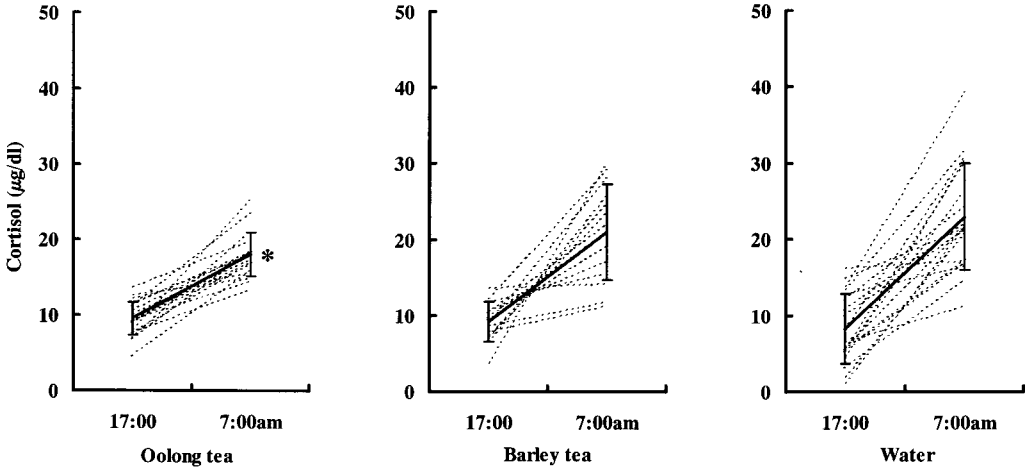

Fig. 4. Effects of Oolong Tea Ingestion on Plasma Cortisol Levels Obtained from Women Loaded with Vigil

Fifty-five healthy Chinese women participated in this study. Subjects received 4 servings of tea daily, the tea bags in each containing 2 g dry weight of oolong tea or barley tea, or else water only, for a period of one week. Plasma cortisol levels obtained from women loaded with vigil in the afternoon (17:00) and morning (7:00) after ingestion of oolong tea, barley tea or water. A significant difference was observed on comparing the change in values between afternoon and morning from the barley tea or water group at $p < 0.05$ as determined with Student's *t*-test.

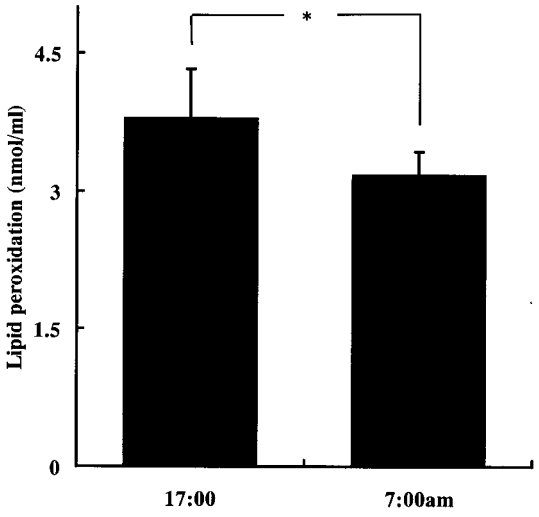

Fig. 5. Plasma Lipid Peroxide Levels in Normal Life Obtained from 55 Healthy Chinese Women

Mean changes of plasma lipid peroxide levels were calculated for afternoon (17:00) and morning (7:00) in normal life. Each bar represents the mean ± S.D. and a significant difference from the control at $p < 0.001$ as determined with Student's *t*-test.

the eyes and headaches compared with the vigil stressed subjects which were given no tea or barley tea in the same way. Additionally, work efficiency within testing sessions was also examined based on calculation performance. Although the error rate was no significant difference among each group in the first test, the error rate of the oolong tea group was lower than that of the drinking water group in total number of errors for the five tests, but there was no obvious difference between the barley tea and water groups. The results indicated positive effects of oolong tea ingestion on test stress reactivity. However, we do not know the mechanism by which oolong tea affects these various symptoms, though it is known that stress limits the supply of energy to organs, which results in the poor utilization of biological energy sources, and leads to fatigue and various physiological disorders. On the other hand, the anti-stress effects of tea have long been recognized.[24] Maruyama *et al.*[25] reported that the ingestion of tea was related with working hours, and this correlation seems to be due to anti-stress effects.[26] In general, such anti-stress effects are thought to be due to the action of caffeine, one of the bioactive components of oolong tea.[27] Bianchi reported that caffeine suppresses adenosine receptors and increases the efflux of calcium ions from nerve terminals.[28] Caffeine promotes energy metabolism by modifying catecholamine release through the stimulation of calcium channels, and thus caffeine may attenuate stress. Arciero *et al.* found that caffeine ingestion elevates the metabolic rate and fatty acid availability through lipolysis in fat cells and the release of catecholamines.[29] The effects of caffeine have been attributed to the adenylylcyclase-cAMP phosphodiesterase cycle.[30] cAMP-dependent protein kinase A in turn activates hormone-sensitive lipase, and this activated enzyme catalyzes the hydrolysis of TG in fat cells.[31]

Barley tea, also called mugicha, is now Japan's

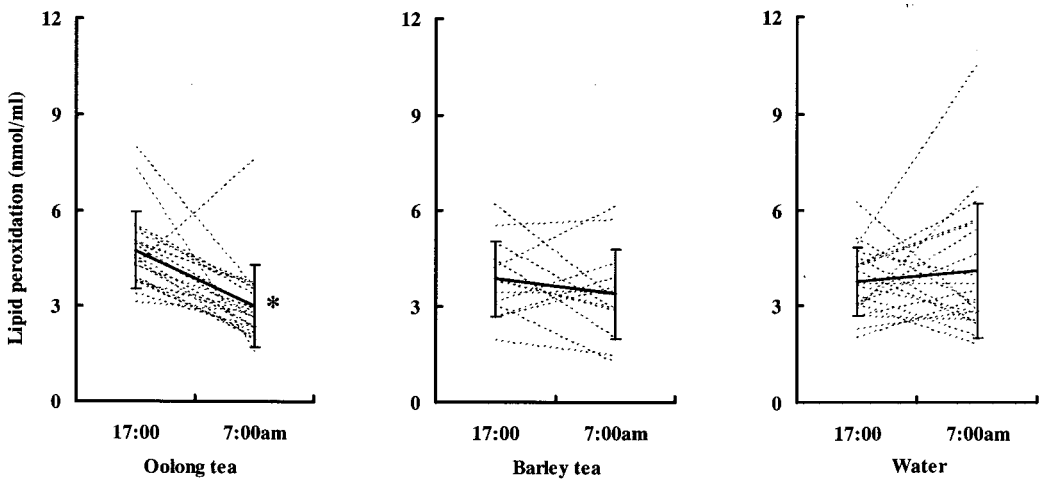

Fig. 6. Effects of Oolong Tea Ingestion on Plasma Lipid Peroxide Levels Obtained from Women Loaded with Vigil

Fifty-five healthy Chinese women participated in this study. Subjects received 4 servings of tea daily, the tea bags in each containing 2 g dry weight of oolong tea or barley tea, or else water only, for a period of one week. Plasma lipid peroxide levels obtained from women loaded with vigil in the afternoon (17:00) and morning (7:00) after ingestion of oolong tea, barley tea or water. A significant difference was observed on comparing the change in values between afternoon and morning from the barley tea or water group at $p < 0.05$ as determined with Student's *t*-test.

most popular soft consumed, and commonly drink it cold during summer. This beverage is not made from the leaves of *Camellia sinensis* L., but brewed from barley kernels. It has been reported that barley tea is beneficial to health.[32] However, the tea has no caffeine and the anti-stress effect was weaker than that of oolong tea.

Cortisol levels in plasma were assessed in response to a vigil mental stressor. The results revealed a significant increase in levels in the barley tea and drinking water groups. In contrast, ingestion of oolong tea resulted in minor changes in the levels. Previous reports have indicated that the blood cortisol level is a good marker of stress because a change in homeostasis is induced by stress.[33] Although cortisol is indispensable for homeostasis including gluconeogenesis, the excess secretion of cortisol induced by stress causes abnormalities in lipid and protein metabolism, and can result in life style-related diseases. However, less attention has been given to the influence of tea ingestion on blood cortisol levels as a stress index. The mechanism responsible for the significant attenuation of the increase in the plasma cortisol level induced by vigil stress is not clear, but our results suggested that oolong tea alleviates stress via a suppressive effect on cortisol production.

It has been widely reported that the blood lipid peroxide level can be used as an index of stress.[34] Usually, there is a balance between the generation and scavenging of active oxygen free radicals in the human body. If there is an imbalance in the mechanism regulating anti-oxidant enzymes such as superoxide dismutase or catalase, excessive amounts of active oxygen radicals can be generated. Previous studies demonstrated that physical or mental stress increases the oxygen concentration in organs, followed by the generation of active oxygen molecules and free radicals.[35] These radicals react with the lipids in the cell membrane. This process causes a chain reaction of lipid peroxide generation in the membrane and seriously damages the cell. The result may be correlated with life-style-related diseases such as cancer,[36] diabetes[37] and atherosclerosis.[38] We measured the mean lipid peroxides of the subjects at baseline before the study, and our results showed that the plasma lipid peroxide level was highest in the afternoon, and the blood lipid peroxide level was lowest in the morning. These observations indicate that lipid peroxide is metabolized during sleep. On the other hand, plasma lipid peroxide levels, which were markedly increased by vigil stress, were significantly lower in the oolong tea group than in the barley tea or water group. These results demonstrate that oolong tea treatment can render lipid peroxides less susceptible to oxidative modification during a stressful vigil stress. It has been reported that the ingestion of polyphenolic compounds, such as (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-gallocatechin gallate GCG and (-)-epicatechin (EC), which are contained in tea, enhanced the anti-oxidative activity in both

humans[39] and animals,[40] and it was discussed that the biological effects of tea are due to the anti-oxidative activities of tea catechins.[41] Green tea and catechins have been reported to have many pharmacolongical properties such as anti-oxidative effects[42] and scavenging effects on free radicals.[43] Although the active substances in oolong tea have not been identified, based on our results, the effects of oolong tea on plasma lipid peroxide levels arise from the anti-stress and antioxidant effects of a variety of polyphenols, caffeine or other active components which are abundant in the tea.

Illness caused by stress has been recognized since ancient times.[44] Moreover, blood cortisol and lipid peroxide levels are important in various stress reactions. Our results clearly demonstrated that ingestion of oolong tea reduced these levels which were markedly increased by vigil stress. Oolong tea may be useful for the prevention of diseases related to stress without adversely affecting appetite or physical fitness.

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