Effect of the Combination of Ginseng, Oriental Bezoar and Glycyrrhiza on Autonomic Nervous Activity and Immune System under Mental Arithmetic Stress

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Summary
Background: Stress reduces physical and mental tolerances (immune potential) of humans and it induces progression of existing illness or causes latent disorders to become active. Thus, the control and suppression of stress plays an important role in the improvement of quality of life and prevention of diseases. Ginseng, oriental bezoar and glycyrrhiza have been used for Kampo (herbal treatment) for thousand years and a number of pharmacological and clinical studies have reported their effects. However, it has not been previously described how the combination of these most commonly used herbs affect mental stress. Objective: This is a randomized, double-blind, placebo-controlled experiment to examine the effectiveness of reducing stress response by taking Kampo. Ten healthy males (mean age 27 ± 1) participated in the study. The effectiveness of stress reduction was assessed by measuring ECG, salivary chromogranin A (CgA), blood glucose, WBC, granulocytes, lymphocytes, NK cell activity, etc. Salivary and blood measurement values of pre- and post-mental arithmetic stress were compared. In addition, ECG measurement values of pre- and mid-mental arithmetic stress were compared. Results: we observed a higher HF power and a lower SNS index, HR, CgA, WBC and granulocytes in the Kampo trial than those in the placebo trial. The HR, HF power and SNS index were changed significantly (p < 0.05) and CgA, WBC and granulocytes tended to show some differences between the two trials (p < 0.1). However, blood glucose, lymphocytes, and NK cell activity showed no significant differences between the Kampo and placebo trials. Conclusions: The result suggests that the Kampo should be useful in reducing mental stress.

Key Words Kampo, stress, salivary CgA

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), suppresses the immune system (3), and can cause acute organ dysfunction (4).

Immune reactions to acute stress suggest the possible involvement of the sympathetic adrenomedullary (SAM) axis in those reactions. This has been supported by evidence that immune reactions to acute stressful tasks disappeared completely during autonomic blockade or surgical processing of sympathetic nerves (5, 6), and the absent immune reactions reappeared by direct stimulation of sympathetic nerves (7). The autonomic nervous system modulation of multicellular organisms was developed to enable cooperation of one cell with others to achieve a single purpose of their behavior. Many cells, therefore, bear adrenergic or cholinergic receptors on the surface. Leukocytes are no exception to this rule (8, 9). Granulocytes and lymphocytes, the two major types of leukocytes that developed from macrophages in phylogeny, possess adrenergic and cholinergic receptors, respectively (10, 11). In this regard, granulocytes are activated in number and function under sympathetic nerve stimulation whereas lymphocytes are so activated under parasympathetic nerve stimulation. Natural killer (NK) cells are a subpopulation of leukocytes that spontaneously express adrenergic receptors (12, 13) and their activity is modulated by sympathetic activity (14, 15). These regulations may be beneficial to protect the body of living beings. However, when the autonomic nervous system deviates too much to one direction, we fall victim to certain diseases. For example, severe physical or mental stress-sympathetic nerve activation-granulocytosis-tissue damage, including collagen diseases, inflammatory bowel diseases, and cancer.

Kampo, composed of specified mixtures of herbs, has been utilized empirically for thousands of years for the treatment of a wide variety of clinical disorders (16). Ginseng, oriental bezoar and glycyrrhiza have been widely used from ancient times as important compo-
nents of Kampo. Kaneo and Nakanishi have recently reported that ginseng has clear anti-stress actions (17). In addition, Morishita et al. (18) demonstrated that a combination of ginseng and oriental bezoar has an anti-stress action and they proved that the result is related to the cooperative effect from oriental bezoar and ginseng. Glycyrrhiza has been used as a harmonizing ingredient in large amounts of Kampo.

Currently, there is no previous study on the effect of the combination of ginseng, oriental bezoar and glycyrrhiza on mental stress. Thus, the purpose of this study is to shed some light on the effect of combined ginseng, oriental bezoar, and glycyrrhiza on mental stress. Therefore, Kampo in the following refers to the combination of ginseng, oriental bezoar and glycyrrhiza.

MATERIALS AND METHODS

Participants. Ten healthy male students with a mean age of 27±1 y from Kyoto University volunteered to participate in this study. None of the subjects was taking any medication, and each subject was instructed to avoid beverages containing alcohol or caffeine and strenuous physical activity on the day before the measurements. The experiment administrator explained the purpose of the experiment, test protocol and bioactivity of Kampo prior to the experiment to all subjects. Then, informed consent to participate in this study was obtained from each subject. The protocol of the study was approved by the ethical committee of Kyoto University Graduate School.

Kampo and placebo. The Kampo capsule at a dose of 2 capsules contained the following ingredients: powered ginseng 250 mg, powered oriental bezoar 50 mg, and powered glycyrrhiza 50 mg. The placebo capsules contained corn starch. Kampo and placebo capsules were kindly supplied by Nitto Pharmaceutical Industries, Ltd.

Stress test. The procedure for inducing mental stress was carried out as follows. The investigator advised the subjects that important measurements were about to be made during which time the subjects would be required to perform the four operations of arithmetic about to be made during which time the subjects would be required to perform the four operations of arithmetic. The subjects were told to chew on the salivettes as regularly as possible. Following the salivary measurement, blood samples were obtained via venipuncture of an antecubital vein. The blood was collected into heparized tubes for measurement of total white blood cells (WBC), lymphocytes, granulocytes, NK cell activity and glucose. Subjects were instructed to remain relaxed during this period. Participants were given a 4-min ECG. A resting period of 60 min was needed to have the drug reach effective plasma levels. Each subject was free to choose a quiet activity for spending the rest period. Magazines and books were made available.

Sixty minutes after taking the Kampo or placebo, instruction was given for the mental arithmetic task and the subjects were allowed to practice the mental arithmetic task for 2 min. Then, samples of stimulated saliva were taken within 2 min. After the salivary measurement, they then continued to do the mental arithmetic task for 2 min. Electrocardiograms were assessed continuously during the mental arithmetic task, as well as salivary measurements. Finally, blood samples were immediately collected again from the subjects. This protocol was carried out on two separate days.

Measures.

Salivary and blood sampling analysis: The salivary chromogranin A (CgA) level was determined by enzyme-linked immunosorbent assay (ELISA), using a previously described method (19). Blood samples were collected in heparinized tubes. The numbers of total WBC, lymphocytes, and granulocytes per sample were determined by standard means and glucose was evaluated by the Glu-DH method (20). NK cell activity was assessed in vitro using a standard Cr51 release assay and K562 erythroleukemic cells as targets (21).

Recording and analysis of physiological variables electrocardiogram: The laboratory room was temperature controlled at 25°C and quiet with a minimization of arousal stimuli. An electrocardiogram was recorded using bipolar surface electrodes attached to the chests of the subjects. The electrocardiogram signal (time constant 0.03 s) was A/D converted at a sampling rate of 1 kHz to obtain RR interval data (trans Era HTB 410, Utah, USA), and stored on a computer. These data were analyzed by frequency-domain analysis. Power spectral analysis assesses sympathetic and parasympathetic activities (22). Higher frequencies of HR variability (HF power, set at 0.15–0.4 Hz) tend to reflect parasympathetic nervous system activity (23, 24). While lower frequencies (LF power, set at 0.03–0.15 Hz) reflect both parasympathetic and sympathetic nervous system activities (22, 25, 26), the ratio of low/high frequencies (L/H ratio, SNS index) represents the activity of the sympathetic nervous system.

Statistical analysis. All data are presented as the mean±SE. All of the statistical analyses were performed with the Statistical Package for Social Sciences (SPSS for Windows, version 11.5, SPSS Inc., Chicago, IL). Significant differences were evaluated by Student’s t test. p
values <0.05 were considered to be statistically significant.

RESULTS

Salivary CgA changes.

Most previous studies have focused on the influences of active stressors, such as mental arithmetic, that elicit cardiovascular reactions characterized by β-adrenergic activation. In the present study, we examined the salivary endocrinological stress marker CgA. CgA is an acidic glucoprotein that is released along with catecholamines from the adrenal medulla and the sympathetic nerve endings (27–29). A recent study has reported that CgA is produced by human submandibular glands and secreted into saliva (30). Salivary CgA has gained attention as a novel stress marker. Whereas cortisol has long been assayed as a stress marker that reflects both mental and physical stress, levels of salivary CgA correlate only with mental stress (31). Figure 1 illustrates the change in salivary CgA concentrations for the pre- and post-metal arithmetic test. CgA showed a tendency to increase in the placebo trial, compared with those in the Kampo trial following the mental arithmetic test. This result suggested that Kampo may suppress increases in the CgA during mental stress (0.89±0.11 vs 1.06±0.14 pmol/mL, p<0.1).

Cardiovascular measurement changes

Table 1 shows changes in cardiovascular measurement pre- and mid-metal arithmetic test.

The data show that HR and SNS activity were significantly increased in the placebo trial and HF power was significantly decreased in the placebo trial, compared with those in the Kampo trial during the mental arithmetic test (HR: 66±4 vs 73±3 bpm, p<0.05; SNS index: 1.17±0.22 vs 1.48±0.19, p<0.05; HF: 722±72.23 vs 594±75.24, p<0.05). However, there were no significant differences in the LF power or total power between the trials. These results suggest that Kampo suppresses increases in HR and SNS activity and decreases the HF power during mental arithmetic.

Blood measures

Data including blood glucose concentration, WBC, granulocytes, lymphocytes, and NK cell activity at the pre-test, post-test are summarized in Table 2. The data show that WBC and granulocytes demonstrated a tendency to increase during the placebo trial, compared with those in the Kampo trial after a mental arithmetic test (WBC: 5,690±414 vs 6,770±575/μL, p<0.1; granulocytes: 44.8±2.3 vs 46.4±2.2%, p<0.1). However, there were no significant effects in blood glu-

| Table 1. Changes in cardiovascular measures [Mean (SE)]. |
|-------------------|-------------------|-------------------|-------------------|
|                  | Pre-test          | Mid-test          |                  |
| HR (bpm)         | Kampo (67 (4))    | Placebo (66 (4))  | Kampo (71 (3))   |
|                  | 1,396 (144)       | 1,452 (91)        | 1,331 (74)       |
|                  | 743 (153)         | 722 (72)          | 635 (79)         |
|                  | 653 (103)         | 730 (76)          | 696 (72)         |
|                  | 1.28 (0.28)       | 1.17 (0.22)       | 1.32 (0.25)      |
|                  |                   |                   | 1.48 (0.19)*     |
| Number of subjects=10. *p<0.05 versus pre-test (Student’s t-test). |

| Table 2. Changes in blood measures [Mean (SE)]. |
|-------------------|-------------------|-------------------|-------------------|
|                  | Pre-test          | Mid-test          |                  |
| Glucose (mg/dL)   | Kampo (88.1 (2.3)) | Placebo (89 (2.8)) | Kampo (88.7 (1.5)) |
| WBC (/μL)         | 5,480 (231)       | 5,690 (414)       | 6,380 (341)       |
| Granulocytes (%)  | 45.3 (1.3)        | 44.8 (2.3)        | 46.1 (2.2)        |
| Lymphocytes (%)   | 46.5 (1.3)        | 46.7 (1.5)        | 46.1 (1.4)        |
| NK cell activity (%) | 22.8 (6.4)     | 21.8 (4.5)        | 23.4 (5.0)        |
| Number of subjects=10. *p<0.1 versus pre-test (Student’s t-test). |
cose concentration, lymphocytes, or NK cell activity between the Kampo and placebo trials. These results suggested that Kampo may suppress increases in the WBC and granulocytes during mental stress.

**DISCUSSION**

The aim of this study was to examine the effects of the Kampo on autonomic nervous activity and immune responses under mental stress in healthy subjects.

In the present study, salivary CgA showed a tendency to be reduced by Kampo. As mentioned above, CgA is released along with catecholamines from the adrenal medulla and the sympathetic nerve endings (27, 28). Therefore, this finding suggests that the Kampo may reduce mental stress responses by suppressing sympathetic nervous activity.

Moreover, the fluctuations in HRV reported in the present study showed higher HF and lower SNS activity in the placebo trial than those in the placebo trial. The HF component in HRV is modulated solely by the parasympathetic nervous system (25, 32) while the SNS index reflects the activity of the sympathetic nervous system (33). Heart rate, which could be increased by increased sympathetic activity and/or decreased parasympathetic activity, showed significant increases in the placebo trial under mental stress. Accordingly, the fluctuation in the HRV index reported in the present study suggests that sympathetic nervous activity in the placebo trial is more dominant than in the Kampo trial under mental stress conditions.

Changes in the number of leukocytes are induced by acute psychological and physical stress (34, 35). It is known that changes in the leukocyte number and distribution are regulated by the autonomic and endocrine systems. Notably, catecholamine and glucocorticoid hormones are considered to be the major mediators. Catecholamine administration, acute psychological stress, and exercise stress induce a leukocytosis acutely, including an increased number of granulocytes and NK cells, and either a mild increase or no change in the number of lymphocytes (34, 36). The results from the early studies raised the question from which sites the newly appearing cells are derived after catecholamine administration. A number of possibilities have been discussed in the literature (37), including the spleen, the marginal zone in blood vessels, bone marrow, lymph, and lungs (38).

We observed a tendency to reduce WBC and granulocytes after taking the Kampo compared with the response to the placebo. Granulocytes, especially neutrophils, have a number of receptor types expressed on their surface, in particular β-adrenergic ones (35). The previous studies have found that activation of β-adrenergic receptors induces a functional rise of neutrophils (39) and increments of WBCs could be interpreted by the increment of neutrophils, which is a type of granulocyte that responds via β-adrenergic receptor activation (40). Thus, our finding also demonstrated that the Kampo administration exhibited a lower sympathetic activity than placebo administration.

Overall, these findings describe autonomic nervous activity, namely sympathetic nervous activity and parasympathetic nervous activity under mental stress response. The important function of the autonomic nervous system (ANS) is to assist the body in maintaining a constant internal environment (homeostasis). The ANS also participates in appropriate coordinated response to external stimuli (41). Accordingly, the response of the ANS to mental stress elicits enhancement of sympathetic nervous activity and/or reduction of parasympathetic nervous activity (42, 43). Prior studies implied that mental stress as a factor precipitates or exacerbates the course of a number of chronic illnesses, such as coronary heart disease (44) and cancer (45).

In conclusion, the main findings in the present study reveal that the Kampo treatment decreased HR, SNS index, WBC and CgA, and increased HF under stress stimulation. We hypothesized that Kampo would suppress the increase in the blood glucose level and NK cell activity which was increased by sympathetic nervous activity, whereas the decrease in the lymphocytes was increased by parasympathetic nervous activity under mental stress stimulation. However, NK cell activity, blood glucose level and lymphocytes did not change significantly. This unexpected result might have been caused by the small number of participants in our study. Because of the stress response to sympathetic nervous activity, the major findings of the present research suggested that the Kampo is likely to relieve stress influences on autonomic nervous activity and the immune system. These results might be beneficial in stress-related diseases, and the pharmacological activities of the Kampo treatment should be investigated clinically. These results may imply that the Kampo treatment may contribute to improvement in diseases related to other organs besides the heart, which deserves further study in the future.

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