Comparison of the Effects of Sho-hange-ka-bukuryo-to and Nichin-to on Human Plasma Adrenocorticotropic Hormone and Cortisol Levels with Continual Stress Exposure

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Sho-hange-ka-bukuryo-to and Nichin-to, traditional Chinese herbal (Kampo) medicines have been used to treat vomiting and nausea. Traditional herbal medicines have frequently been used in the empirical treatment. Some patients who take these medicines have no organic disease but have conditions classified as non-ulcer dyspepsia (NUD). To determine the pharmacological effects of Sho-hange-ka-bukuryo-to, Nichin-to, and the two herbs (*Pinelliae Tuber* and *Zingiberis Rhizoma*, both of which are included in Sho-hange-ka-bukuryo-to and Nichin-to), we examined the effects of these medicines on the plasma levels of adrencorticotropic hormone (ACTH) and cortisol under stress conditions by repetitive blood sampling. After a single administration of Kampo medicine or a placebo, venous blood samples were taken before and 20—240 min after administration. A single administration of Sho-hange-ka-bukuryo-to caused significant suppression of an increase in plasma ACTH-immunoreactive substance (IS) levels at 120 to 180 min and tended to suppress increases in plasma cortisol levels at 240 min, compared with the response to a placebo. A single administration of Nichin-to caused significant suppression of increases in plasma ACTH-IS levels at 120 min compared with a placebo group, but had no effect on plasma cortisol levels. *Pinelliae Tuber* had no significant effects in plasma ACTH-IS or cortisol, but *Zingiberis Rhizoma* significantly suppressed the increase of ACTH-IS (120 min) and cortisol (180 min). These medicines have a modulatory effect on the hypothalamo-pituitary-adrenal (HPA) axis and autonomic nervous function. These effects might be beneficial in stress-related disease and suggest that this medicine has clinical pharmacological activity.

Key words stress; Kampo; adrenocorticotropic hormone (ACTH); cortisol

Traditional Chinese herbal (Kampo) medicines (Sho-hange-ka-bukuryo-to, Nichin-to, Rikkunshi-to, Hange-shashin-to, *etc.*) have frequently been used in empirical treatment of chronic hypofunction of the gastrointestinal system. Some patients who take these Kampo medicines have no organic disease such as peptic ulcer; reflex esophagitis or gastric cancer but have a condition classified as non-ulcer dyspepsia (NUD).[1] Most NUD patients tend to have depressive and psychosomatic conditions and are exposed to continual affective stress.[2] That continual stress causes abnormalities in the hypothalamo-pituitary-adrenal (HPA) axis and autonomic nervous function.[3,4]

Sho-hange-ka-bukuryo-to, a Kampo medicine regulating gastrointestinal function, has been used to treat nausea, vomiting, acute and chronic gastritis and upper gastrointestinal abnormalities (gastric atony). It has been especially widely used to treat hyperemesis of pregnancy. Nichin-to also has been used to treat nausea and vomiting, and clinical difference between these two medicines is slight. *Pinelliae Tuber* and *Zingiberis Rhizoma* are herbs, both of which are included in Sho-hange-ka-bukuryo-to and Nichin-to, with antiemetic activity.[5,6] Therefore, the pharmacological effects of the two medicines might be due to the herbs.

ACTH is a peptide containing 39 amino acids, and ACTH-immunoreactive substance (IS) is found in tissues other than the pituitary gland (*i.e.*, brain, adrenal gland, gastrointestinal tract, pancreas, thyroid gland and placenta).[7] The secretion of ACTH is controlled by the circadian rhythm mechanism and negative feedback from plasma cortisol and neurogenic stimulation. The peptide has secretory action of glucocorticoid and rises under stress.

Cortisol, commonly used to indicate the level of stress, is secreted by the zona fasciculate of the adrenal cortex and its secretion is dependent on the ACTH level.

In general, venipuncture for blood sampling is postulated to be a stress factor that can increase circulating ACTH and cortisol levels, *etc.*[8,9] Repetitive blood sampling places subjects under artificial stress and venipuncture as a stressor is useful for the evaluation of the pharmacological effects of drugs.[10—12] Naito *et al.* reported other Kampo medicines (Rikkunshi-to, Hange-shashin-to, Hange-koboku-to) regulated plasma ACTH and cortisol levels under stress.[13] These medicines also commonly included *Pinelliae Tuber* and *Zingiberis Rhizoma*.

To determine whether the pharmacological effects of Sho-hange-ka-bukuryo-to and Nichin-to on stress-related hormone levels (ACTH and cortisol) under continual stress, and to determine the contribution of the herbs to the pharmacological effects of Kampo, we compared the plasma ACTH-IS and cortisol levels after administration of these Kampo medicines and related herbs.

MATERIALS AND METHODS

Materials Sho-hange-ka-bukuryo-to (EK-21, lot 1XC31), prepared as a 1.7 g dried powder extract of *Pinelliae Tuber* (6.0 g), *Zingiberis Rhizoma* (2.0 g) and *Hoelen* (5.0 g) were kindly supplied by Kanebo Co., Ltd. (Tokyo, Japan). Nichin-to (TJ-81, lot 21010041), prepared as a 3.0 g dried powder extract of *Pinelliae Tuber* (5.0 g), *Glycyrrhizae Radix* (1.0 g), *Hoelen* (5.0 g), *Zingiberis Rhizoma* (1.0 g) and *Aurantii Nobilis Pericarpium* (4.0 g) were supplied by Tsumura Co., Ltd. (Tokyo). *Pinelliae Tuber* extract was purchased from Nippon Funmatsu Yakuhin (Osaka), and *Zingiberis Rhizoma* extract

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was purchased from Sainokiyu (Osaka). Placebo was the additive of the above formulations, *Pinelliae Tuber* extract and *Zingiberis Rhizoma* extract alone.

Synthetic human ACTH (1—24) was purchased from the Peptide Institute (Osaka). Antiserum to human ACTH (A516/R1H) was purchased from Biogenesis (Newfields, U.K.), and the TDx Cortisol assay kit from Dainabot (Tokyo). All other reagents were analytical reagent grade from commercial sources.

Subject Five healthy male volunteers (nonsmokers), aged 24—29 years (median 28 years), 55—68 kg (median 62 kg), participated in the study. Each subject received information on the scientific purpose of the study and gave written informed consent. The study was approved by the ethical committee of Oita Medical University. The subjects did not receive any medication for at least one month prior to the study, and fasted for 2 h before the study commenced and during the experiments.

Study Schedule Sho-hange-ka-bukuryo-to at a dose of 6.0 g, Nichin-to at a dose of 7.5 g, *Pinelliae Tuber* extract (5.0 g), *Zingiberis Rhizoma* extract (2.5 g) or placebo was administered orally with 100 ml water. Each subject was administered these drugs at intervals of one month. The dose of medicines was the maximum given dose as a daily dose in clinical therapy. Venous blood samples (10 ml) were taken from a forearm vein before and at 20, 40, 60, 90, 120, 180 and 240 min after administration of the drug (eight times). All subjects ate lunch at 11:45—12:00, and the study was carried out from 14:00 until 18:00. When blood samples were taken at intervals of 120 min, sampling was performed at 14:00, 16:00 and 18:00 without administering a test medicine.

Enzyme Immunoassay (EIA) of ACTH The blood samples were placed in chilled tubes containing 500 kallikrein inhibitor units/ml of aprotinin and 1.2 mg/ml of EDTA. After centrifugation, plasma samples were diluted fivefold with 4% acetic acid (pH 4.0) and loaded onto a C18 reversed-phase cartridge (Sep-Pak C18; Millipore Corp., Milford, MA, U.S.A.). After washing with 4% acetic acid, plasma peptides were eluted with 70% acetonitrile in 0.5% acetic acid (pH 4.0). Elutes were concentrated by spin-vacuum evaporation, lyophilized and stored at −40 °C until assayed.

EIA for ACTH was performed as previously described.[14] The assay was performed by a delayed addition method. Separation of bound and free antigen was performed on an anti-rabbit IgG (55641, ICN Pharmaceuticals, Inc., Ohio, U.S.A.) coated immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark). Human ACTH (1—24) was conjugated with β-galactosidase (Boehringer Mannheim, Mannheim, Germany) by *N*-(ε-maleimidocaproyloxy)-succimide according to the method of Kitagawa *et al.*[15] The concentration of ACTH was expressed as ACTH (1—24), which has the biological activity of ACTH. The EIA for ACTH was specific and highly sensitive to detection limits of 2.0 fmol/ml.

Determination of Plasma Cortisol Levels Plasma cortisol levels were measured using a fluorescence polarization immunoassay. The detection limit of cortisol was 0.64 μg/dl. This method shows minimal cross-reactivity with the endogenous steroids (11-deoxycortisol [9.9%], corticosterone [6.3%] and others [<3%]).[16]

Statistical Analysis ACTH-IS levels in plasma are expressed as mean±S.D. (ng/ml). Cortisol levels in plasma are expressed as a concentration of mean±S.D. (μg/ml). Comparison of mean values was made by Mann Whitney *U* test and $p<0.05$ was considered statistically significant.

RESULTS

Effect of Sho-hange-ka-bukuryo-to on ACTH-IS and Cortisol Levels The plasma ACTH-IS level–time profile after a single oral administration of Sho-hange-ka-bukuryo-to is shown in Fig. 1a. The dotted line shows the levels of ACTH-IS in samples at 120-min intervals (4.3±2.0 pg/ml at 120 min and 5.3±2.2 pg/ml at 240 min). There was a showed significant suppression of increases compared with placebo at 120 min, which reflected the effects of repetitive blood sampling. At 120 Sho-hange-ka-bukuryo-to also caused a significant suppression of increases in ACTH-IS between 120—180 min (5.1±1.2 pg/ml at 120 min and 4.2±1.6 pg/ml at 180 min), compared with the response of the placebo group (9.4±2.5 pg/ml at 120 min and 8.9±1.6 pg/ml at 180 min).

Figure 1b shows the plasma cortisol level–time profile after administration of Sho-hange-ka-bukuryo-to. The dotted line shows the levels of cortisol in samples at 120-min intervals (4.5±1.5 μg/dl at 120 min and 2.9±0.5 μg/dl at 240 min). There was a significant suppression of increases compared with placebo at 240 min, which reflected the effects of repetitive blood sampling. Sho-hange-ka-bukuryo-to had no significant effect on plasma cortisol levels; however, this medication showed 76.2% (240 min) inhibition of placebo cortisol levels.

Effect of Nichin-to on ACTH-IS and Cortisol Levels Figures 2a and b show plasma ACTH-IS and cortisol level–time profile after a single oral administration of

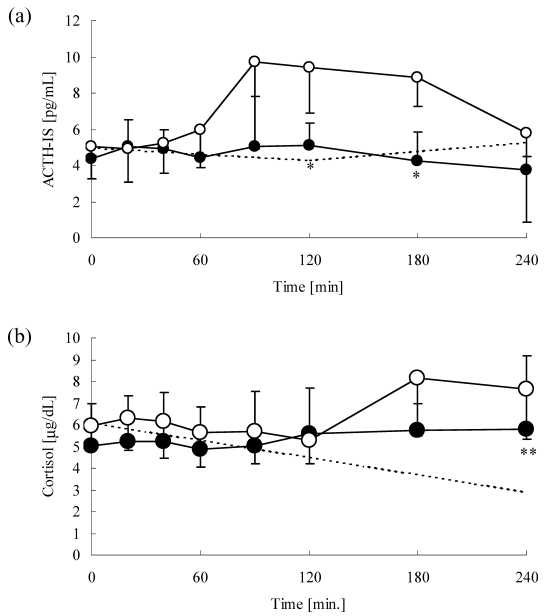

Fig. 1. Effect of Sho-hange-ka-bukuryo-to (●) or Placebo (○) on Plasma ACTH-IS (a), Cortisol (b) Levels

Each value represents the mean±S.D. of concentrations in five volunteers. *$p<0.05$ and **$p<0.1$ compared with placebo.

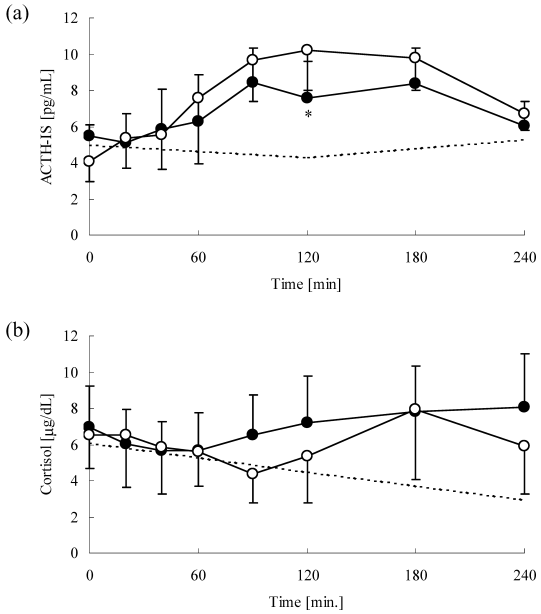

Fig. 2. Effect of Nichin-to (●) or Placebo (○) on Plasma ACTH-IS (a), Cortisol (b) Levels

Each value represents the mean±S.D. of concentrations in five volunteers. $*p<0.05$ and $**p<0.1$ compared with placebo.

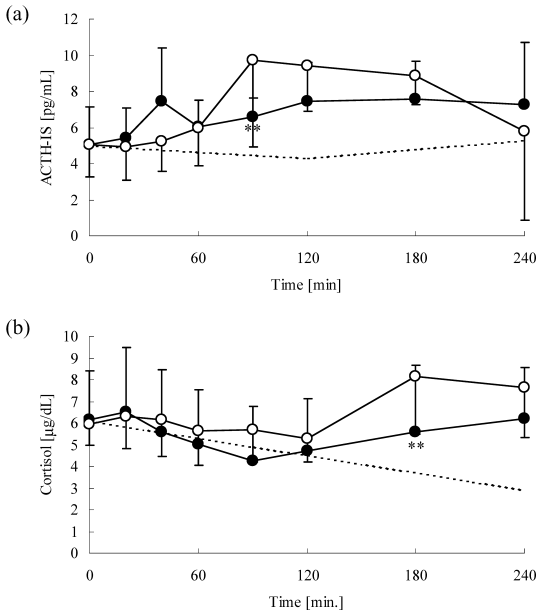

Fig. 3. Effect of *Pinelliae Tuber* Extract (●) or Placebo (○) on Plasma ACTH-IS (a), Cortisol (b) Levels

Each value represents the mean±S.D. of concentrations in five volunteers. $**p<0.1$ compared with placebo.

Nichin-to. Nichin-to caused significant suppression of increases in ACTH-IS at 120 min (7.6±2.0 pg/ml), compared with the response of the placebo group (10.20±2.2 pg/ml), although it had no significant effect on plasma cortisol levels.

Effect of Herbs (*Pinelliae Tuber* and *Zingiberis Rhizoma* Extract) on ACTH-IS and Cortisol Levels The plasma ACTH-IS and cortisol levels after the administration of *Pinelliae Tuber* extract are shown in Figs. 3a and b. *Pinelliae Tuber* extract had no significant effect on plasma these levels, but it showed 67.8% (90 min) inhibition of placebo

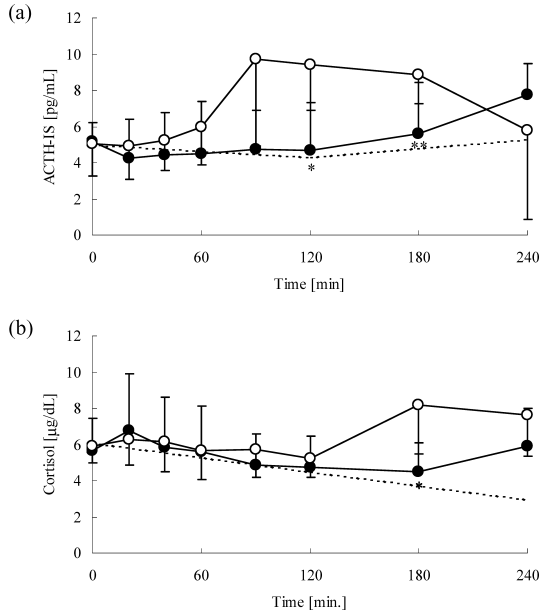

Fig. 4. Effect of *Zingiberis Rhizoma* Extract (●) or Placebo (○) on Plasma ACTH-IS (a), Cortisol (b) Levels

Each value represents the mean±S.D. of concentrations in five volunteers. $*p<0.05$ and $**p<0.1$ compared with placebo.

ACTH-IS levels and 68.3% (180 min) inhibition of placebo cortisol levels.

Figure 4a shows the effects of *Zingiberis Rhizoma* extract on plasma ACTH-IS levels. The extract caused a significant suppression of increases in ACTH-IS at 120 min (4.7±2.7 pg/ml) compared with the response of the placebo group (9.4±2.5 pg/ml) and showed 63.2% (180 min) inhibition of placebo ACTH-IS levels.

Figure 4b shows the effects of *Zingiberis Rhizoma* extract on plasma cortisol levels. This extract caused a significant suppression of increases in cortisol at 180 min (4.5±1.6 μg/dl) compared with the response of the placebo group (8.2±2.3 μg/dl).

DISCUSSION

Plasma ACTH levels are regulated by the two major pathways of circadian rhythm and negative feedback. Repetitive blood sampling raised ACTH-IS levels in plasma compared with sampling at intervals of 120 min in volunteers who received placebo. Those effects of placebo on ACTH are assumed to result from mental and/or physiological stress in volunteers due to repetitive blood sampling. Volunteers from whom samples were taken at intervals of 120 min are assumed to have been under less stress. Sho-hange-kabukuryo-to and Nichin-to suppressed the increases in ACTH-IS levels compared to placebo. The herbs contained in these Kampo medicines might play a role in regulation of ACHT release. In both Kampo medicines used this study, *Pinelliae Tuber* and *Zingiberis Rhizoma* are common ingredients. It is empirically known that *Zingiberis Rhizoma* has antiemetic activity[6] and *Pinelliae Tuber* has both emetic and antiemetic activity.[5,17] But it is reported that when treated together with *Zingiberis Rhizoma*, the emetic activity of *Pinelliae Tuber* vanishes.[18] In this study, *Zingiberis Rhizoma* had modulatory

effects on plasma ACTH-IS and cortisol levels, but *Pinelliae Tuber* had no effect on plasma ACTH-IS, perhaps because its usual modulatory effect was canceled by its emetic active component. Sho-hange-ka-bukuryo-to and Nichin-to, which contain *Pinelliae Tuber* and *Zingiberis Rhizoma*, had modulatory effects on plasma ACTH-IS levels. The suppression effect of Sho-hange-ka-bukuryo-to is stronger than that of Nichin-to, and this might be due to the quantity and/or ratio of the two herbs.

Cortisol is commonly used to indicate the level of stress. Samples taken at intervals of 120 min showed a decreasing trend in cortisol levels, corresponding with circadian rhythm. In general, plasma cortisol levels are high in the morning and gradually decrease from morning to afternoon.[19] Both Sho-hange-ka-bukuryo-to and Nichin-to tended to suppress increases in cortisol levels compared to placebo. Only *Zingiberis Rhizoma* extract, which is included in both Kampo medicines, had a significant effect on plasma cortisol levels. Further studies are needed to elucidate why the modulatory effect of *Zingiberis Rhizoma* was eliminated when mixed with other herbs.

Sho-hange-ka-bukuryo-to and Nichin-to had modulatory effects on the HPA axis from the viewpoint of ACTH and cortisol. Both medicines might influence corticotropin-releasing hormone (CRH) or ACTH, which exists upstream on the HPA axis compared with cortisol. In this study, repetitive blood sampling resulted in increases in ACTH (120, 180 min) and cortisol (180, 240 min) levels. Changes in plasma ACTH levels were more rapid than those in cortisol, and CRH levels might change before the changes in ACTH and cortisol. With regard to the effects of Kampo and herbs for the HPA axis, Bupleuri Radix-containing Kampo medicines stimulate the secretion and synthesis of pituitary ACTH, and the effects are mediated by hypoyhalamic CRH.[20—22]

Most NUD patients are exposed to continual affective stress. The continual stress causes abnormalities in the HPA axis and autonomic nervous function. Kampo medicines which regulate gastrointestinal function have been used empirically to treat abnormalities of the gastrointestinal system such as NUD. Based on the empirical effects, the effects of some gastrointestine-targeting Kampo medicine is assumed to be due to changes in the levels of gut-motor regulatory hormones (*i.e.*, somatostatin, gastrin, motilin and vasoactive intestinal peptide.[23—27] In addition to this mechanism, Sho-hange-ka-bukuryo-to and Nichin-to were found to affect the HPA axis in this study. However, the effects of these Kampo medicines on the HPA axis were caused by repetitive blood sampling. It is suspected that cause of the stress from repetitive blood sampling is the same as that causing of NUD. Therefore it is necessary to investigate the effects of these Kampo medicines on the HPA axis and autonomic nervous system in patients with a condition such as NUD.

In conclusion, Sho-hange-ka-bukuryo-to and Nichin-to regulated plasma ACTH levels under stress. These modulatory effects might be beneficial in stress-related disease and the pharmacological activities of these medicines should be researched clinically.

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