Effects of Domperidone on Human Plasma Levels of Motilin, Somatostatin, Gastrin, Adrenocorticotrophic Hormone and Cortisol

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Domperidone, an upper gastrointestinal function regulatory medicine, has recently been evaluated for its clinical usefulness in the treatment of stress and depression. We examined the effects of domperidone on the plasma levels of motilin-immunoreactive substance (IS), somatostatin-IS, gastrin-IS, adrenocorticotrophic hormone (ACTH)-IS, and cortisol under stress conditions by repetitive blood sampling. After a single oral administration of domperidone (30 mg), the plasma domperidone level was highest (58.6 ± 6.4 ng/ml) in the sample taken 40 min after administration, after which the plasma level fell. Peak plasma motilin-IS levels (23.1 ± 1.4 pg/ml) were achieved 40 min after administration of domperidone ($p < 0.01$ vs. placebo), and returned to baseline levels within a further 40 min. Plasma somatostatin-IS levels (13.0 ± 1.2 pg/ml) increased 60 min after administration of domperidone ($p < 0.01$ vs. placebo). Plasma gastrin-IS levels did not change significantly. These results suggest that the pharmacological effects of domperidone on gastrointestinal functions are closely related to changes in motilin-IS and somatostatin-IS levels. Domperidone significantly suppressed increases in plasma ACTH-IS and cortisol levels compared with the response to a placebo. These modulatory effects might be beneficial in stress-related diseases and suggest that this medicine has clinical pharmacological activity.

Key words domperidone; adrenocorticotrophic hormone (ACTH); cortisol; stress; motilin; somatostatin

Domperidone is an antinauseant and gastrokinetic drug which has been reported to be effective for treating functional gastrointestinal disorders such as dyspepsia, gastroesophageal reflux, nausea and vomiting. Domperidone also has recently been evaluated for its clinical usefulness in stress and depression. This medicine has frequently been used in the treatment of chronic hypofunction of the gastrointestinal system.^{1,2} Some patients who take this medicine do not have organic diseases such as peptic ulcer, reflux esophagitis, and gastric cancer but have conditions classified as non-ulcer dyspepsia (NUD). Most NUD patients tend toward depressive and psychosomatic conditions and are exposed to continual affective stress.³ This continual stress causes abnormalities in the hypothalamo-pituitary-adrenal (HPA) axis and autonomic nervous function.^{4,5} Some abnormalities of gastrointestinal function are presumed to result from changes in hormone levels. However, the mechanism is not clear.

The gastrointestinal peptide motilin is found in specific endocrine cells of the upper small intestine of hogs.⁶ This peptide strongly stimulates fundic pouch activity⁷ and plays an important physiological role in intestinal contractility, particularly in the fundus and antral of the pouch of the stomach. It is one of the most important factors controlling the regular occurrence of phase III interdigestive migrating contractions.^{8,9}

Somatostatin is present in high amounts in the stomach.¹⁰ This peptide inhibits the secretion of motilin and gastrin, and it also inhibits gastric, duodenal, and biliary motility.¹¹

Gastrin was first detected in extracts of pyloric antral mucosa.¹² The most potent actions of gastrin are the stimulation of both gastric acid secretion and antral motility.¹³

Adrenocorticotrophic hormone immunoreactive substances (ACTH-IS) are found in tissues other than the pituitary gland (*i.e.* brain, adrenal gland, gastrointestinal tract, pancreas, thy-

roid gland, and placenta). The secretion of ACTH is affected by mechanisms of circadian rhythm and negative feedback by blood cortisol, and neurogenic stimulation.¹⁴ Plasma levels of ACTH increase under stress and the peptide has the secretomotor action of glucocorticoid.⁴ Cortisol, commonly used to indicate the level of stress, is secreted by the zona fasciculata of the adrenal cortex and its secretion is dependent on the ACTH level.

In general, venipuncture for blood sampling is believed to be a stress factor that can increase circulating levels of ACTH, cortisol, and other substances.^{15,16} Repetitive blood sampling places subjects under artificial stress, and venipuncture as a stressor is useful for evaluation of the pharmacological effects of drug.¹⁷

The purpose of this study was to determine the effects of domperidone on plasma levels of motilin-IS, somatostatin-IS, gastrin-IS, ACTH-IS, and cortisol in healthy individuals.

MATERIALS AND METHODS

Subjects Five healthy male volunteers, aged 25–31 (median 28) years and weighing 54–70 (median 65.1) kg, participated in the study. Each subject received information about the scientific purpose of the study, which was approved by the Ethics Committee of Oita Medical University, and gave informed consent. No subject received any medication for two weeks preceding the test and no stimulator of gastrointestinal motility, except for domperidone, was administered to any subject during the study.

Study Schedules Domperidone (Nauzelin tablets, Kyowa Co., Ltd., Tokyo, Japan) was given orally as a single dose of 30 mg with water to the 5 volunteers. Two weeks later, placebo (lactose tablets) was given orally to the volunteers. Venous blood samples (10 ml) were taken from a forearm vein before, and at 20, 40, 60, 90, 120, 180, and 240 min

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after administration. The study was carried out from 14:00 to 18:00 to avoid the effects of lunch. All subjects ate lunch at 12:00. In the study in which 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.

Determination of Domperidone Concentration in Plasma Domperidone was measured by a modification of the method of Yamamoto *et al.*¹⁸⁾ A 0.1 ml plasma sample was mixed with 0.1 ml water containing 3 ng of propranolol as an internal standard, 0.1 ml of 0.01 M NaOH and 5 ml of chloroform. The mixture was shaken for 20 min and centrifuged at $3000 \times g$ for 10 min. Then, 4 ml of the organic phase was transferred to another tube and 2.5 ml of 0.01 M HCl was added. After the mixture was shaken and centrifuged, 2 ml of the upper aqueous phase was transferred to another tube and 0.05 ml of 1 M NaOH and 5 ml of chloroform were added. The mixture was shaken for 20 min and then centrifuged. Four milliliters of the lower organic phase was transferred to another tube and evaporated to dryness and reconstituted in a 100 μ l mobile phase, and 5 ml of the solution was injected onto a chromatograph. HPLC was carried out using a guard-Pak precolumn (Cosmosil 5C18-AR, Nacalai Tesque, Kyoto, Japan, 10 mm $\times$ 4.6 mm I.D.) and a C18 column (Cosmosil 5C18-AR, Nacalai Tesque, 150 mm $\times$ 4.6 mm I.D.) at 25 °C and UV absorbance was detected at 282 nm for excitation and 328 nm for emission. The HPLC consisted of a model 600E pump system (Millipore). A mixture of 0.02 M phosphate buffer–methanol (45 : 55, v/v) was used as the mobile phase at a flow rate of 1 ml min⁻¹. The recovery of domperidone in plasma using this extraction procedure was >93%.

Preparation of Plasma Extracts The blood samples were placed in chilled tubes containing aprotinin 500 kallikrein inhibitor units/ml (Trasylol®, Bayer Co., Ltd., Leverkusen, Germany) and EDTA 1.2 mg/ml (Wako Pure Chemical Industries, Ltd., Osaka, Japan). After centrifugation (1670 $\times g$, 4 °C, 20 min), plasma samples were diluted with 4% acetic acid, pH 4.0, and loaded onto Sep-Pak C18 cartridges (Millipore). After washing with 4% acetic acid, pH 4.0, each peptide in plasma was eluted with 70% acetonitrile in 0.5% acetic acid, pH 4.0. The eluates were concentrated by spin-vacuum evaporation, lyophilized, and stored (–40 °C) until a highly sensitive enzyme immunoassay (EIA) was performed. The recoveries of plasma motilin-IS, somatostatin-IS, gastrin-IS, and ACTH-IS were all >93% with this extraction procedure (data not shown).

EIAs for Motilin-IS, Somatostatin-IS, Gastrin-IS, and ACTH-IS EIA for motilin-IS was performed as previously described.¹⁹⁾ Antiserum (Y121) was obtained from Peptide Institute, Inc. (Osaka, Japan). Motilin was labeled with β -D-galactosidase (Boehringer Mannheim Corp., Mannheim, Germany). EIA was performed by the delayed-addition method. Separation of bound and free materials was performed using an immunoplate (Nunc-Immuno Module Maxisorp F8, InterMed, Denmark) coated with anti-rabbit IgG (55641, ICN Pharmaceuticals, Inc., Ohio, U.S.A.). The EIAs for somatostatin-IS,²⁰⁾ gastrin-IS,²¹⁾ and ACTH-IS²²⁾ were essentially the same, except for using the antisera for gastrin (A600/R1B; Biogenesis, Ltd., U.K.), somatostatin (RA-08-108; Cambridge Research Biochemicals, Cambridge, U.K.), and ACTH (A516/RIH; Biogenesis, Ltd., U.K.), respectively.

The EIAs for motilin-IS, somatostatin-IS, gastrin-IS, and ACTH-IS were specific and highly sensitive to detection limits of 0.80, 0.04, 0.10, and 2.0 fmol/ml, respectively. The recovery rates of these human plasma peptides between the proposed detectable ranges with these EIA ranged from 94.8 to 98.7%. The reproducibility (CV%) for human plasma with these peptide EIAs ranged from 2.2 to 3.1% of innerassay ($n=5$), and from 4.1 to 5.5% of intraassay ($n=10$) comparisons.

Determination of Plasma Cortisol Levels Plasma levels were measured using a fluorescence polarization immunoassay. The detection limit for cortisol was 6.4 ng/ml. This method shows minimal cross-reactivity with endogenous steroids (11-deoxycortisol [9.9%], corticosterone [6.3%], and others [$<3\%$]).

Data Analysis The area under the plasma concentration-time curve from 0 to 240 min after administration ($AUC_{0-240 \text{ min}}$) was calculated using the trapezoidal method. All values are expressed as the mean $\pm$ S.D. Comparison of mean values was made by analysis of variance and Dunnett's test. A value of $p < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Domperidone improves gastrointestinal motility and reduces gastric stasis, gastroesophageal reflux, and gastrointestinal dysfunction.¹⁾ In the first study, gut-regulated peptide (motilin, somatostatin and gastrin) levels, which regulate gastrointestinal function, were examined to determine their relation to domperidone. The levels of motilin, somatostatin, and gastrin in samples at 120-min intervals did not change significantly compared with placebo (data not shown). The table shows the $AUC_{0-240 \text{ min}}$ of the peptides after the administration of domperidone or placebo.

The profiles of average plasma domperidone concentrations plotted against time after oral administration of the drug are shown in Fig. 1. The plasma level was highest (58.6 ± 6.4 ng/ml) in the 40 min sample.

The highest plasma motilin-IS levels (23.1 ± 1.4 pg/ml) were recorded 40 min after oral administration of domperidone, and had declined to the baseline level by 60 min. Domperidone caused significant increases in motilin-IS at 20, 40, and 60 min compared with placebo (Fig. 2a). The release of motilin ($AUC_{0-240 \text{ min}}$) increased by 10.2% with domperidone (Table 1). Marzio *et al.* suggested that dopamine caused significant increases in plasma motilin levels, and pretreatment with domperidone completely prevented the dopamine

Fig. 1. Plasma Domperidone Levels after Oral Administration of 30 mg of the Drug

Each value represents the mean $\pm$ S.D. of levels in 5 male volunteers.

Fig. 2. Plasma Motilin-IS (a), Somatostatin-IS (b), and Gastrin-IS (c) Levels after Oral Administration of Domperidone (●) or Placebo (○)

Each value represents the mean $\pm$ S.D. of levels in 5 male volunteers. ** $p < 0.01$ compared with placebo.

Table 1. $AUC_{0-240 \text{ min}}$ of Peptide Release (pg/ml min) after Administration of Domperidone or Placebo to Healthy Volunteers

	Placebo	Domperidone
Motilin	2630.5 $\pm$ 59.0	2897.3 $\pm$ 76.6**
Somatostatin	1351.5 $\pm$ 186.7	1917.9 $\pm$ 103.4**
Gastrin	6419 $\pm$ 319.5	6518 $\pm$ 595.6
ACTH	1176.9 $\pm$ 85.9	1013.4 $\pm$ 32.6**
Cortisol	35862 $\pm$ 1876.3	31242 $\pm$ 1629.7**

Each value represents the mean $\pm$ S.D. of concentrations in five tests. ** $p < 0.01$ compared with placebo.

effect.²³⁾ However, Funakoshi *et al.* reported that domperidone caused significant increases in plasma motilin levels.²⁴⁾ In their study, motilin-IS levels after domperidone administration were enhanced by several pathways. Fox *et al.*²⁵⁾ and Poitras *et al.*²⁶⁾ reported that muscarinic receptors are present on the membrane of motilin-secreting cells, and that acetylcholine is a major regulator of motilin release. Our findings suggest that domperidone may stimulate motilin cells *via* muscarinic receptors. The elevated motilin level also appeared to indicate that domperidone might enhance motor

activity in the small intestine, including phase III contractions of the migrating motor complex (MMC).^{8,9)} After oral administration of domperidone, the plasma levels of somatostatin-IS were the highest at 13.0 ± 1.2 pg/ml in the 60-min sample (Fig. 2b). Marzio *et al.* suggested that dopamine did not affect somatostatin levels.²³⁾ However, octreotide, a somatostatin analogue, has a longer half-life than somatostatin and stimulates MMC-like activity in the small intestine.^{27,28)} The release of somatostatin ($AUC_{0-240 \text{ min}}$) increased by 45.1% with domperidone (Table 1). Somatostatin, which is widely distributed in the gastrointestinal tract, participates in the control of gut motility by exerting both inhibitory and stimulating influences.^{29,30)} The plasma gastrin levels after administration of domperidone are shown in Fig. 2c. The drug had no significant effect on plasma gastrin-IS level; generally, gastrin is secreted as a result of stimulation of the gastric mucosal G cells.³¹⁾

Furthermore, the time to peak plasma domperidone levels after administration of the drug was 40 min. Single administration of domperidone caused a significant increase of plasma motilin and somatostatin in accordance with the plasma domperidone levels. Based on the results of this study, we conclude that domperidone may improve gastrointestinal motility by significantly increasing motilin-IS and somatostatin-IS levels in plasma.

Domperidone also has modulatory effects on the HPA axis and autonomic nervous function.^{4,5)} Some abnormalities of gastrointestinal function are caused by obstruction of the HPA axis and autonomic nervous system and by changes in hormone levels. We examined the effects of the gastrointestinal function regulatory medicine, domperidone, on the plasma levels of ACTH-IS and cortisol under stress conditions. Banky *et al.* reported that domperidone administration markedly decreased prolactin levels in animals subjected to restraint stress.³²⁾

The plasma ACTH-IS level-time profile when blood was sampled at intervals of 120 min is shown in Fig. 3a (dotted line). The levels of ACTH-IS in samples at 120-min intervals (3.9 ± 0.5 pg/ml at 120 min, 4.4 ± 0.4 pg/ml at 240 min) showed suppression of increase compared with placebo, which reflected the effects of repetitive blood sampling. Domperidone significantly suppressed increases in ACTH-IS at 90 and 120 min compared with the response of the placebo. The ACTH-IS levels in volunteers after the administration of domperidone were almost the same as those in samples taken at intervals of 120 min. The release of ACTH decreased by 13.5% with domperidone (Table 1).

Fig. 3b (dotted line) shows the plasma cortisol level-time profile when blood samples were taken at intervals of 120 min. Placebo caused significant increases in cortisol at 240 min compared with the levels when samples were taken at intervals of 120 min (133.2 ± 20.2 ng/ml), and no significant changes were seen at 120 min (136.8 ± 18.8 ng/ml). Domperidone significantly suppressed increases in cortisol at 180 and 240 min compared with the response with placebo. The release of ACTH decreased by 13.7% with domperidone (Table 1).

Imrich *et al.* reported that domperidone did not affect the levels of ACTH and cortisol.³³⁾ In another study, plasma ACTH levels were regulated by the two major pathways of circadian rhythm and negative feedback.¹⁴⁾ Repetitive blood

Fig. 3. Plasma ACTH-IS (a), and Cortisol (b) Levels after Oral Administration of Domperidone (●) or Placebo (○)

Each value represents the mean $\pm$ S.D. of levels in 5 male volunteers. * $p < 0.05$ and ** $p < 0.01$ compared with placebo.

sampling raised ACTH-IS levels in plasma compared with sampling at intervals of 120 min in volunteers administered placebo. These effects of placebo on ACTH are assumed to result from mental and/or physiological stress in volunteers due to repetitive blood sampling.^{15,16} Volunteers in whom samples were taken at intervals of 120 min are assumed to be under less stress. Domperidone suppressed the increases in ACTH-IS levels compared with placebo.

Cortisol is commonly used to indicate the levels of stress. In general, plasma cortisol levels are high in the morning and gradually decrease from morning to afternoon.³⁴ Domperidone suppressed the increase in cortisol levels compared with placebo. Those levels showed the same circadian tendency.

Domperidone had a modulatory effect on the HPA axis from the viewpoint of ACTH and cortisol.^{4,5} However, there are very few reports on the effects of domperidone on the levels of these hormones.³⁵ This medicine might influence corticotropin-releasing hormone (CRH) or ACTH, which exist upstream on the HPA axis compared with cortisol. In this study, repetitive blood sampling resulted in increases in ACTH (90, 120 min) and cortisol (180, 240 min) levels. The changes in plasma ACTH levels were more rapid than those in cortisol, and CRH levels might change before the changes in ACTH and cortisol.

Most NUD patients are exposed to continual affective stress. The continual stress causes abnormalities in the HPA axis and autonomic nervous functions.^{4,5} Gastrointestinal function regulatory medicine has been used empirically to treat abnormalities of the gastrointestinal system such as NUD. Based on the empirical effects, gastrointestinal targeting medicine has been elucidated from gut motor regulatory hormone levels (motilin, somatostatin and gastrin). The empirical effects were assumed to be due to changes in the

levels of somatostatin, motilin, or gastrin. In addition to this mechanism, which is related to gastrointestinal regulatory hormones, domperidone was found to affect the HPA axis in this study. However, in our study, the effects of gastrointestinal function regulatory domperidone on the HPA axis were caused by repetitive blood sampling. It is therefore necessary to examine the effects of this medicine on the HPA axis and autonomic nervous system in patients with conditions such as NUD.

In this study, domperidone regulated plasma ACTH and cortisol levels under stress. These modulatory effects might be beneficial in stress-related disease and the pharmacological activities of these medicines should be investigated clinically.

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