Effect of Saiko-ka-ryukotsu-borei-to on Amylase Activity in Mice

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The effects of Saiko-ka-ryukotsu-borei-to (SRBT), a Chinese medicinal prescription, on mouse serum amylase activity were investigated in vivo. SRBT was found to not only dose- and/or time-dependently augment amylase activity, but also to increase α-amylase protein content and soluble starch metabolic activity. These results provide a rational basis for the clinical use of SRBT that may accompany disease therapy.

Key words Saiko-ka-ryukotsu-borei-to; α-amylase; SDS-polyacrylamide gel electrophoresis (SDS-PAGE); serum; mouse

Previously we reported on the direct influence of Chinese medicinal prescriptions on amylase activity in mouse plasma in vitro. Interestingly, a few prescriptions seem to augment the activity of α-amylase in plasma by more than 70%. Here, we examined the influence of Saiko-ka-ryukotsu-borei-to (SRBT), which strongly augments plasma amylase activity in vitro, on serum amylase activity in vivo. The mechanisms were also examined by electrophoretic methods.

MATERIALS AND METHODS

Materials and Prescription Chopped crude drugs, defined according to the Jpananese Pharmacopeia (14th edition), were purchased from Nakai-koshindo (Kobe, Japan). The reagents used for SDS-PAGE were purchased from Bio-Rad Co. Ltd. (Hercules, CA, U.S.A.). Other drugs (analytical reagent grade) were purchased from Nacalai Tesque (Kyoto, Japan). The Chinese medicinal prescription was prepared according to the prescription for a one-day dose as described in the literature. The following prescription was used as SRBT: Bupleuri Radix (6.0 g), Pinelliae Tuber (4.0 g), Poria (3.0 g), Cinnamomi Cortex (3.0 g), Scutellariae Radix (2.5 g), Zizyphi Fructus (2.5 g), Zingiberis Rhizoma (0.5 g), Ginseng Radix (2.5 g), Fossilia Ossis Mastodi (2.5 g), Ostereae Testa (2.5 g), Rhei Rhizoma (1.0 g). The prescription (1-d dose) was decocted in a beaker with 600 ml of water by boiling for 40 min over an electric heater (600 W) and filtered through absorbent cotton, followed by concentration in vacuo and freeze-drying. The yield was 4.88 g for SRBT.

Animals Male ddY mice aged 7—8 weeks and weighing 28—32 g (Nihon SLC, Hamamatsu, Japan) were housed in groups of 6 in plastic cages with free access to food (Japan CLEA; CE-2, Tokyo) and water. They were kept in a room maintained at an ambient temperature and humidity (25±5°C, 55±5%) under a day/night regime (day 7:00—19:00 and night 19:00—7:00). All animals were maintained in the laboratory for a minimum of 1 week prior to the start of the experiment and fasted for 18 h before drug administration.

Assay of Serum Amylase Activity and Blood Sugar Content Cardiac blood was collected without any anesthesia from the mice by decapitation followed by centrifuging (12000×g, 20 min) to prepare serum. The amylase activity was measured according to the Caraway method using a kit (Amylase-Test Wako, Wako Pure Chemical Industries Co., Ltd., Japan) as described previously. The activity (amylase unit) was calculated as (1−B/A)×100, where A is the activity of the enzyme without test solution and B the activity of the enzyme with test solution. The serum glucose content was estimated according to the O-toluidine-boric acid method using a kit (Glucose-Test Wako, Wako Pure Chemical) as previously described.

SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a Mini-Protein III apparatus (Bio-Rad, Hercules, CA, U.S.A.). The serum samples (collected from individual mice, n=7—9, protein content was adjusted to 2.0 mg/ml) were re-suspended in a sample buffer containing 62.5 mM Tris–HCl (pH 6.8), 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 0.00625% (W/V%) bromophenol blue, and then boiled for 4 min followed by loading onto a 7.5% acrylamide/bisacrylamide (29 : 1) separating gel. Electrophoresis was carried out at a constant voltage of 200 V for 40 min.

Quantification of Serum α-Amylase Protein The amylase protein separated by SDS-PAGE was quantified using PC-associated image analysis. The SDS-PAGE gels were digitized using a PC-scanner (EPSON ES-2200, EPSON Co. Ltd., Nagano, Japan) operating on the image acquisition and analysis program, L Process V2.0 and Image Gauge V4.0 (FUJI PHOTOFILM Co., Ltd., Tokyo, Japan). The signal from each band examined in the SDS-PAGE gel, expressed in optical density units, was converted to amylase unit equivalents using the equation obtained from the linear regression of data for the amylase standards. Proteins were stained using a Rapid Stain Coomassie brilliant blue kit (Nacalai Tesque, Kyoto). The serum protein contents were measured by the method of Bradford.

Quantification of Starch-Metabolite Activity The starch metabolite activity observed by non-reducible SDS-PAGE was quantified in the same manner as mentioned above. Non-reducible SDS-PAGE was applied for the assay of the starch-metabolite activity on the gel. Briefly, the samples were re-suspended in a sample buffer without 2-mercaptoethanol followed by electrophoresis under the same conditions. Additional soluble starch was added to 34 mM NaCl and 100 mM phosphate buffer, pH 7.0, to make a 10 g/l suspension for gel incubation. The concentrated stock iodine solution for staining contained 18 mM KI and 5.1 mM I2 and was added to a Rapid Stain Coomassie brilliant blue kit (Nacalai Tesque, Kyoto). The starch-metabolite activity was measured by the iodine color reaction as described previously.

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stained in 30 ml of diluted iodine solution.

**Statistical Analysis** The results are expressed as the mean±S.E. (n=7—9) following a one-way analysis of variance for repeated measurements with the overall data being used to assess statistical significance. Differences between the individual mean values in various groups were analyzed by the Mann–Whitney U-test. A difference was considered significant at p<0.05.

**RESULTS AND DISCUSSION**

The influences of SRBT on mouse serum amylase activity were investigated by measuring the disappearance of 660 nm-absorbance caused by amylase activity. Cardiac blood was collected at each sampling time after drug administration. As indicated in Fig. 1, SRBT (600 or 1200 mg/kg, p.o.) exhibited dose- and/or time-dependent augmentation of amylase activity. Significant augmentation by 122% and 137% at 1 and 3 h, respectively, after a dose of 600 mg/kg was observed, while a similar phenomenon was only found at 1 h after a dose of 1200 mg/kg. Figure 2 shows the change in serum glucose level after SRBT (600 or 1200 mg/kg, p.o., 1 h) administration. The serum glucose level increased significantly by 17% at a dose of 600 mg/kg, and by 16% at 1200 mg/kg.

SRBT produces an increase in the level of α-amylase protein as well as its enzymatic activation. Figure 3 shows a representative picture of SDS-PAGE and the results of quantification. The amount of α-amylase protein in 2 mg/ml of total serum protein tended to increase after 60 or 180 min of SRBT (1200 mg/kg, p.o.) administration by 109 or 110%, respectively. The changes in non-reducible SDS-PAGE for starch-metabolite activity were also examined and the result of quantification is shown in Fig. 4. At that time, the mean optical density of the α-amylase band stained by iodine solution was diminished in comparison with the background, indicating amylase activity. After 60 or 180 min of SRBT (1200 mg/kg, p.o.) administration, further diminution of the mean density was observed by 119 or 140%, respectively, indicating significant activation of α-amylase activity at 180 min.

Previously we reported that SRBT augmented plasma amylase activity in vitro, suggesting the influence of that differ from the known results. This study has examined the details of the activation in serum amylase in vivo. We found that mouse serum α-amylase activity augmented by SRBT may depend on modification of the amount of amylase protein. Amylase gene expression is principally affected by glucocorticoids in vivo. Corticosterone regulates amylase synthesis directly in rat pancreas, and the mechanism was explained in terms of the alteration of mRNA level or gene transcription. On the other hand, SRBT was reported to exhibit anti-stress activity in rodents, investigated by a stress-
induced increase in serum corticosterone levels in mice, and stress-induced disruption of a glucocorticoid negative feedback system in rats. Numerous studies have assessed the positive relationship between not only acute stress stimuli and glucocorticoids, but also acute stress stimuli and a hyperglycemic response via the central nervous system. Hypothalamic noradrenergic neuronal activity plays an important role in blood glucose regulation. Noradrenaline release from the medial basal hypothalamus was rapidly and markedly increased under acute swimming stress execution, while acetylcholine release from the lateral hypothalamic area was not, indicating the serum glucose level was also significantly increased. Moreover, during acute stress stimuli, the increased level of glucose was important for maintaining the availability of ATP to muscles or the central nervous system. Furthermore, SRBT contains huge amounts of triterpenoids as the ingredients of its constructed crude drugs; Bupleuri Radix, Poria, Zizyphi Fructus and Ginseng Radix. There are many reports concerning the relation between triterpenoids and glucocorticoids (for a review, see ref. 17). One example is that saikosaponin d increases corticosterone-releasing factor mRNA levels in rat hypothalamus. Consequently, it may be assumed that SRBT ingredients, especially triterpenoids, play important roles in the results described in this paper. That is to say, SRBT might be a possible activator of serum amylase activity, suggesting it should contribute to the anti-stress activity. Further investigations are necessary to clarify the contributions of the ingredients contained in this Chinese medicinal prescription in order to determine the mechanism of activity.

REFERENCES