Administration of Perilla Oil Coated with Calshell Increases Glucagon-Like Peptide Secretion

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Recently, we found that unsaturated long-chain fatty acids (such as α-linolenic acid) promote the secretion of glucagon-like peptide-1 (GLP-1) via G protein-coupled receptor GPR120, which is expressed predominantly in the colon. In order to ensure that the triglycerides or free fatty acids, such as α-linolenic acid, reach the distal intestinal tract effectively, we developed a Calshell technique. Following single treatment of Calshell perilla oil powder, the GLP-1 secretion level was significantly higher than following vehicle treatment, 120 min after treatment. Next, we examined the effects of long-term Calshell perilla oil powder treatment on GLP-1 secretion. Plasma GLP-1 level of Calshell perilla oil powder treatment was significantly higher than of vehicle treatment for 1, 14, 28 and 56 d. We thereby demonstrated for the first time the utility of Calshell oil powder treatment for effective and sustainable GLP-1 secretion. The Calshell technique is apparently useful as a drug delivery system, since Calshell unsaturated oil powder is protected from gastric acid, reaches enteroendocrine cells in the gastrointestinal tract, and then induces effective incretin secretion.

Key words Calshell; perilla oil; glucagon-like peptide-1; insulin; mouse

Recent studies have shown that elevated triacylglycerol concentrations, especially in the postprandial state, may be associated with increased risk of coronary heart disease. It has been established that fish oil suppresses plasma triacylglycerol concentrations in both postprandial and fasting states in humans and experimental animals. Enhanced rate of fatty acid oxidation in the liver appears to be a crucial factor for the hypotriglyceridemic effect of fish oil in the fasting state. It has been reported that dietary fats rich in α-linolenic acid (α-LA) such as linseed and perilla oils increased hepatic fatty acid oxidation rate through both mitochondrial and peroxisomal pathways in the fasting state.

The activity of peroxisomal oxidation systems is known to be enhanced by administration of various substances that induce a hypotriglyceridemic effect.

Moreover, it has been found recently that some triglycerides and free fatty acids (FFAs) also act as signaling molecules. Administration of a fatty-acid mixture directly into the ileum of rats is known to stimulate incretin secretion by the L-cells. Recently, we found that unsaturated long-chain FFAs (such as α-LA) promote secretion of glucagon-like peptide-1 (GLP-1) via G protein-coupled receptor GPR120 in STC-1 enteroendocrine cells and in vivo. We have also found that GPR120 is expressed predominantly in the colon. Our present study indicates that, among nutrients, FFAs can act as stimuli. In particular, FFAs in the colon potentially promote GLP-1 secretion, and thereby lead to a reduction in blood glucose levels even in diabetic animals.

Several materials have been developed as drug delivery systems (chitosan, polysaccharide, calcium pectinate, etc.). We developed a Calshell technique, so that triglycerides or FFAs, such as α-LA, could reach the distal intestinal tract effectively. Calshell is a microcapsule wherein the food compositional material is protected by a calcium shell, and it displays gastric acid tolerance.

In this study, using Calshell technology, we examined whether acute and long-term treatment with triglycerides, administered to mice with or without Calshell, affects GLP-1 secretion. For oil treatment, we used perilla oil, which contains a high ratio of α-LA.

MATERIALS AND METHODS

Chemicals Perilla oil (Ohta Oil Mill, Co. Ltd., Okazaki, Aichi, Japan) was used for as a test reagent, and polyethylene glycol (PEG) 400 (Sigma, St. Louis, MO, U.S.A.) was used as a vehicle solution for administration to mice.

Preparation of Perilla Oil Coated in Calshell Perilla oil and calcium powder are added to water and agitated at a high rate to form an oil in water emulsion and, at the same time, electrostatically absorb calcium microparticles around oil droplets, using the Ultra-High Speed Mixing System (T.K. ROBOMICS) apparatus (PRIMIX Corporation, Osaka Japan). Subsequently, the resultant solution containing the calcium shells are spray-dried to obtain Calshell perilla oil powder.

Biodegradation Assay of Calshell Perilla Oil Powder Estimation of Calshell perilla oil powder biodegradation was performed according to the Japanese Pharmacopoeia. In brief, Calshell perilla oil powder was suspended in experimental gastric juice (70 mM HCl, 0.2% NaCl, pH 1.2) or experimental intestinal juice (23.6 mM NaOH, 50 mM KH2PO4, pH 6.8), and was shaken at 100 rpm in 37 °C for 30, 60 and 90 min. After shaking, these solutions were centrifuged, and oil content in supernatant was quantified by the method as previously reported. Data shows the ratio of the released oil to total oil in Calshell perilla oil powder, as α-LA equivalent.

Animal Experiment. Acute Treatment Male C57BL/6J mice (8 weeks old) were purchased from SLC (Hama-
matsu, Japan). This study was approved by the Kyoto University Animal Care and Use Committee. The animals fasted for at least 18 h prior to experiments, and were anaesthetized with sodium pentobarbital (60 mg/kg). Perilla oil and Calshell perilla oil powder (3 μmol/100 μl total administration) were administered orally. Blood samples were collected from the portal vein, before administration and 15, 30, 60, 90, 120, 180, 360, and 600 min after administration, and centrifuged (n=6). Plasma obtained from blood samples was used for measurement of GLP-1.

**Long-Term Treatment** Male C57BL/6J mice (8 weeks old) were purchased from SLC (Hamamatsu, Japan). The animals were fed with a high-fat rodent chow diet (Quick Fat, Crea Japan, Osaka, Japan), and had free access to food and tap water. The animals were maintained in a temperature-controlled room (23 °C) on a 12-h light dark cycle. Oral administration (3 μmol/100 μl total administration) was repeated every day for 56 d (pellira oil, Calshell perilla oil powder and vehicle: n=8). After 1, 14, 28 and 56 d, the animals were made to fast for at least 18 h prior to treatment, and were anaesthetized with sodium pentobarbital (60 mg/kg). Two hours after oral administration of perilla oil, Calshell perilla oil powder or vehicle, blood samples were obtained from the portal vein, and were centrifuged. Plasma obtained from blood samples was used for measurement of GLP-1 and insulin levels.

**GLP-1 and Insulin Levels** Plasma levels of GLP-1 (GLP-1 ELISA Kit, Wako Pure Chemical, Osaka, Japan) and insulin (Revis Insulin Kit, Shibayagi, Maebashi, Japan) were measured, using blood samples.

**Statistical Analysis** Results were expressed as mean±S.E.M. Statistical significance was evaluated by ANOVA, and statistical significance was defined as p<0.05.

**RESULTS AND DISCUSSION**

We have reported that α-LA induces GLP-1 secretion via GPR120, and that α-LA is most effective with respect to colon for GLP-1 secretion in the gastrointestinal tract. We consider it necessary for α-LA to be delivered to colon, in order to stimulate GLP-1 secretion. We recently developed a Calshell technique. Calshell is a microcapsule wherein the food compositional material is protected by a calcium shell, and it displays gastric acid tolerance. In Fig. 1, Calshell perilla oil powder was resistant to experimental gastric juice. Therefore, we think that Calshell perilla oil powder is effective for delivery of oil to colon. In this study, we administered single and long-term treatment with perilla oil, which contains a high ratio of α-LA, to mice with or without Calshell, in order to investigate effects on GLP-1 secretion.

First, we administered single treatment with perilla oil and Calshell perilla oil powder, in order to examine the effect on GLP-1 secretion. GLP-1 secretion level was significantly higher in perilla oil treatment than in vehicle PEG treatment at 30, 60 and 120 min after treatment (Figs. 2A, B). Since the results were consistent with our previous study concerning α-LA treatment, it appears that perilla oil is a potent reagent for stimulation of GLP-1 secretion. Moreover, with Calshell perilla oil powder treatment, GLP-1 secretion level was significantly higher than with vehicle treatment at only 120 min after treatment (Figs. 2A, B). However, area under the curve (AUC) for GLP-1 secretion level was higher from 0 to 120 min after administration of perilla oil and Calshell perilla oil powder than after administration of vehicle (Fig. 2C). These results suggest that Calshell perilla oil powder delays secretion of GLP-1. Furthermore, AUC from 0 to 600 min was higher in Calshell perilla oil powder administration than in administration of vehicle and normal perilla oil (Fig. 2D). These results suggest that Calshell perilla oil powder not only delays but also increases secretion of GLP-1.
Next, we examined the effects of long-term Calshell perilla oil powder treatment on GLP-1 secretion in mice that had been administered a high fat diet, as a model of metabolic syndrome. Plasma GLP-1 levels were significantly higher in Calshell perilla oil powder treatment for 1, 14, 28 and 56 d than in vehicle treatment (Fig. 3A). These results suggest that long-term Calshell perilla oil powder treatment induces GLP-1 secretion. Interestingly, while administration of a high fat diet for 56 d induced hyperinsulinemia, hyperinsulinemia was not detected after perilla oil and Calshell perilla oil powder treatment had been administered for 56 d (Fig. 3B). It has been reported that exogenously administered GLP-1 exerts a glucose-dependent insulinotropic effect on pancreatic β-cells, and that, on the other hand, in a GLP-1 analog treatment study, GLP-1 leads to improved glucose homeostasis, while also alleviating insulin resistance. We surmise that increased plasma GLP-1 level on normal perilla oil and Calshell perilla oil powder administration improves insulin resistance induced by high fat diet.

In conclusion, we demonstrate for the first time the utility of Calshell oil powder treatment for achieving effective and sustainable GLP-1 secretion. It appears that the Calshell technique is a useful drug delivery system, in that the unsaturated oil in Calshell is protected from gastric acid, reaches enteroendocrine cells in the gastrointestinal tract, and induces effective incretin secretion.

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