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Short Communication

Effects of sitagliptin on plasma incretin concentrations after glucose administration through an esophagostomy tube or feeding in healthy cats

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ABSTRACT

We investigated the effect of sitagliptin, a dipeptidyl peptidase 4 inhibitor, on plasma incretin concentrations after glucose administration through an esophagostomy tube or feeding in healthy cats. Six cats were used for the glucose administration experiment and 5 cats were used for the feeding experiment. Glucose administration through an esophagostomy tube increased plasma glucagon-like peptide 1 (GLP-1) concentrations by 6-fold, whereas plasma glucose-dependent insulinotropic polypeptide (GIP) concentrations did not change. Feeding increased both plasma GLP-1 concentrations by 1.5-fold and GIP concentrations by 4.6-fold. Sitagliptin was administered through an esophagostomy tube (25 and 50 mg per cat) in the glucose administration experiment and orally (25 mg per cat) in the feeding experiment. Sitagliptin treatment potentiated the GLP-1 response to glucose by 1.5-fold ($P < 0.05$). In addition, postprandial plasma GLP-1 concentration was higher by 2-fold when sitagliptin was administered ($P < 0.05$). In contrast, administration of sitagliptin did not affect plasma GIP concentrations after glucose administration or feeding. Sitagliptin enhanced insulin secretion following glucose administration by 1.5-fold ($P < 0.05$); however, it did not influence the plasma glucose concentration. Furthermore, sitagliptin had no effect on the postprandial plasma glucose and insulin concentrations. In conclusion, this study provides no evidence that sitagliptin is beneficial for management of feline diabetes mellitus.

1. Introduction

Incretins are enteroendocrine cell-derived hormones that stimulate insulin secretion [1]. They include glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are released from intestinal endocrine L and K cells, respectively, in humans, rodents, and cats [1–3]. Secretion of GLP-1 and GIP is stimulated by meal ingestion or glucose, fatty acids, amino acids, and dietary fiber; however, certain differences exist among species [2]. For example, the most potent stimulator of GIP secretion in humans is fat, whereas carbohydrates are the most potent stimulators in rodents and pigs [2]. Both GLP-1 and GIP stimulate glucose-dependent insulin secretion from pancreatic beta cells in rodents and humans [2,4]. The effect of incretins on insulin secretion has been observed in healthy cats, although it was minimal compared with that in other species [5]. Lipids and amino acids were potent stimulators of both GLP-1 and GIP, whereas glucose increased only GLP-1 concentration in cats [5]. Other effects

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of incretins, such as beta cell proliferation, inhibition of glucagon secretion, and suppression of glucose production in the liver, are also reported in rodents [2,6,7].

Dipeptidyl peptidase 4 (DPP-4) is a serine protease that inactivates incretins [8]. It has been detected in widespread organs including small intestine, biliary tract, exocrine pancreas, spleen, and brain in rodents and humans [8]. Because DPP-4 rapidly metabolizes GLP-1 and GIP, the half-life of GLP-1 and GIP in circulation is <2 min [9,10]. Therefore, degradation by DPP-4 is one of the major factors that affect activity of the incretins.

After a meal, DPP-4 inhibitors increase active incretin concentrations and potentiate insulin secretion in humans [11]. DPP-4 inhibitors, such as sitagliptin and vildagliptin, have been used as monotherapy in patients with type 2 diabetes or in combination with metformin, sulfonylurea, or thiazolidinedione [6]. Because spontaneous diabetes in cats is known to be similar to human type 2 diabetes [12], it is possible that DPP-4 inhibitors are beneficial for the management of feline diabetes mellitus. However, the effect of DPP-4 inhibitors in cats has not been extensively examined. In the present study, the effect of sitagliptin on blood incretin and insulin concentrations was investigated in healthy cats after glucose administration through an esophagostomy tube or feeding.

2. Materials and methods

2.1. Animals

All procedures involving the cats were performed at Tottori University and were approved by the Animal Research Committee of Tottori University (11-T-110). Six normal cats (4 spayed females and 2 castrated males; mean age, 8.0 ± 1.1 yr; body weight, 3.7 ± 0.3 kg) were used for the glucose administration experiment and 5 normal cats (3 spayed females and 2 castrated males; mean age, 8.0 ± 1.2 yr; body weight, 3.7 ± 0.4 kg) were used for the feeding experiment. Each cat had a body condition score of 3 (5-point scale). All cats were confirmed to be healthy by physical examination, complete blood count, and serum biochemical tests.

2.2. Glucose administration experiment

One week before the experiment, a 5-Fr esophagostomy tube was placed under sedation after intramuscular injection of 50 μg/kg of medetomidine (Domitor; Nippon Zenyaku Kogyo, Fukushima, Japan), followed by antagonization with intramuscular injection of 125 μg/kg atipamezole (Antisedan; Nippon Zenyaku Kogyo). The ostomy sites were kept clean with povidone–iodine gel (Isodine Gel 10%; Meiji Seika Pharma, Tokyo, Japan).

The cats were withheld from food overnight but were allowed free access to water throughout the experiment. They were divided into 3 groups of 2 cats in each group to receive sitagliptin (25 mg, 50 mg, or clean water as placebo) according to a Latin square design with a 1-wk washout period between experiments.

Sitagliptin (Januvia; Banyu Pharmaceutical, Tokyo, Japan) (25 or 50 mg per cat) or clean water as placebo was administered through the esophagostomy tube at 9:00 AM. A 50% glucose solution (2 g/kg; Otsuka Pharmaceutical, Tokyo, Japan) was administered through the esophagostomy tube at 11:00 AM. Blood samples were collected from the medial saphenous vein at 9:00 AM, 11:00 AM, 11:30 AM, 12:00 PM, 1:00 PM, and 3:00 PM to measure plasma glucose, insulin, GLP-1, and GIP concentrations.

2.3. Feeding experiment

The cats were withheld from food overnight but were allowed free access to water throughout the experiment. They were divided into 2 groups receiving sitagliptin (25 mg and placebo) according to a Latin square design with a 1-wk washout period between experiments.

Sitagliptin (25 mg per cat) or clean water as placebo was administrated orally at 9:00 AM. The dose rate of sitagliptin was decided from data obtained from the oral glucose administration experiment. A standard dry food (Science Diet; Hill's, Topeka, KS, USA) was fed at 11:00 AM. The amount of food was calculated using the formula: 0.5 × daily energy requirements (1.1 × 60 × body weight [kilogram]) [13]. Blood samples were collected from the medial saphenous vein at 9:00 AM, 11:00 AM, 1:00 PM, 3:00 PM, and 5:00 PM to measure plasma glucose, insulin, GLP-1, and GIP concentrations.

2.4. Assays

All blood samples were collected into ice-cold ethylenediaminetetraacetic acid tubes containing a DPP-4 inhibitor (Millipore, Billerica, MA, USA). Plasma was immediately separated by refrigerated centrifugation (6,000× *g*, 5 min) and stored at −30°C until glucose, insulin, GLP-1, and GIP concentrations were measured. Plasma glucose concentration was measured using dry chemistry methods (DRI-CHEM; FUJIFILM, Tokyo, Japan). Plasma insulin, GLP-1, and GIP concentrations were determined using the Feline Insulin Measurement Kit (Morinaga Institute of Biological Science, Kanagawa, Japan) [14,15], the Glucagon-Like Peptide-1 (Active) ELISA Kit (Millipore) [16], and the Human GIP (Total) ELISA Kit (Millipore) [5], respectively. The assays for GLP-1 and GIP were originally developed for humans. Although amino acid sequences of GLP-1 and GIP have not been determined in cats, the amino acid sequences of feline GLP-1 and GIP predicted from genome sequences (XP_003990856.1 and XP_003996790.1, respectively) indicated 100% and 98% homology with humans, respectively. These ELISA were also validated for feline samples in our laboratory. Measured value of insulin after serial dilution of plasma showed good linearity in the concentration range of 0.4 to 3.4 μg/mL (R^2 value of 0.9996 with a slope of 0.973 and a y-intercept of 0.0824). Recovery after addition of feline insulin ranged from 91% to 115%. The inter- and intra-assay coefficient of variation for insulin assay were 11.4% and 5.4%, respectively. Measured value of GLP-1 after serial dilution of plasma showed good linearity in the concentration range of 2.1 to 34.4 pM (R^2 value of 0.9991 with a slope of 0.967 and a y-intercept of 1.434). Recovery after addition of human GLP-1 ranged from 85% to 113%. The inter- and intra-assay coefficient of variation for GLP-1 assay were 9.2% and