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Constitutive increase in active GLP-1 levels by the DPP4 inhibitor ASP4000 on a new meal tolerance test in Zucker fatty rats

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ABSTRACT

Glucagon-like peptide-1 (GLP-1), an incretin hormone, is essential for the regulation of insulin secretion and glucose homeostasis. GLP-1 is rapidly degraded by dipeptidyl peptidase 4 (DPP4); therefore, DPP4 inhibitors are considered to be a novel class of oral antihyperglycemic agents. These agents are currently under development as treatments for type 2 diabetes. Normally, oral glucose tolerance tests are used for evalating glucose-lowering efficacy, but the augmentation of active GLP-1 via DPP4 inhibition in this test was transient. It has been proposed that the secretion of GLP-1 is regulated by the rate of entry of nutrients into the small intestine; therefore, we have established the new meal tolerance test method using solid diet. This model allows for the continuous monitoring of active GLP-1 secretion after food intake. ASP4000 is an orally effective inhibitor of DPP4 that greatly augments meal-stimulated circulating levels of active GLP-1 constitutively and improves hyperglycemia. Acarbose improved glucose tolerance in the test to a degree similar to that of the DPP4 inhibitor. Our new meal tolerance test is useful for evaluating postprandial hyperglycemia and could be an excellent model for studying the secretion of active GLP-1 via the inhibition of DPP4.

1. Introduction

Hyperinsulinemia is the physiological response that maintains glucose homeostasis in the insulin-resistant organism. Many endogenous substances augment glucose-stimulated insulin secretion and may play a role in β-cell compensation in insulin resistance. Extensive research done during the past three decades has resulted in the identification of two incretin hormones associated with the hyperinsulinemia seen with obesity and diabetes. Glucose-dependent insulinotropic polypeptide (GIP, also known as gastric inhibitory polypeptide) was first discovered in the 1970s in extracts of the upper intestine. Subsequently, it was found to potentiate glucose-stimulated insulin release. Over a decade later, glucagon-like peptide-1 (GLP-1) was identified as an additional cleavage product produced in the lower intestine as a result of proglucagon gene cloning. Incretin stimulation accounts for 30–60% of postprandial insulin release [1]; this incretin effect is abnormal in patients with diabetes [2]. GLP-1 is intimately involved in the regulation of glucose-dependent insulin secretion and has a number of other effects that also promote glucose tolerance [3]. GLP-1 acts via specific receptors on β-cells to increase insulin biosynthesis and secretion, thereby maintaining the ability of the pancreas to regulate the disposal and storage of energy following nutrient absorption [4]. The study of gastrointestinal hormone secretion has relied primarily on the measurement of circulating levels of hormones in the systemic or portal blood [5,6]. Plasma concentrations of GLP-1 are relatively low compared with those of GLP and other gasterointestinal hormones, such as peptide YY and ghrelin [7,8]. In the blood, GLP-1 is rapidly inactivated by the aminopeptidase dipeptidyl peptidase 4 (DPP4). This enzyme cleaves the first two amino acids, leaving an inactive form. Hence, the half-life of biologically active GLP-1 is only 1.5–2 min, and the biological effect is even shorter [9]. Consequently, less than 10% of the active peptide secreted enters the arterial blood stream to reach various target organs. A major drawback in studying the mechanisms of GLP-1 secretion is that the sensitivity of assays currently available for its measurement is at the limits of detection in most settings. The only exception is when the secretion of GLP-1 is driven to high levels in experimental paradigms [10]. Thus, it has been difficult to determine plasma GLP-1 concentrations in rodent models under usual fasting and feeding conditions.

DPP4 is a 766-amino acid peptidase that is membrane-associated and found in numerous tissues as well as T-cells, B-cells, and natural killer cells. DPP4 also exists as a soluble circulating form in plasma [11]. The prevention of GLP-1 inactivation by DPP4 inhibition is currently being actively explored as a novel approach to the treatment of type 2 diabetes [12]. We discovered ASP4000, which

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is a new, potent, selective DPP4 inhibitor [13], in our laboratory. We demonstrated that a single oral administration of ASP4000 suppressed plasma DPP4 activity, and then reduced the glucose level by increasing the active GLP-1 and insulin levels in OGTT in Zucker fatty rats. The purpose of the present study was to examine the effect of ASP4000 in the new meal tolerance test (MTT) model and determine its usefulness as a model for type 2 diabetes using acarbose, α-glucosidase, which is categorized as a drug for lowering postprandial hyperglycemia.

2. Materials and methods

2.1. Chemicals

ASP4000 [(2*S*)-1-{[(1*R*,3*S*,4*S*,6*R*)-6-hydroxy-2-azabicyclo[2.2.1]hept-3-yl]carbonyl}-2-pyrrolidinecarbonitrile hydrochloride] was synthesized in our laboratories. H-Gly-Pro-AMC was purchased from Bachem AG (California, USA), and AMC was purchased from MP Biomedicals (California, USA). Acarbose was purchased from AAPIN Chemicals Ltd. (Oxfordshire, United Kingdom).

2.2. Animals

Male Zucker lean and fatty rats were purchased from Charles River Japan (Yokohama, Japan) at the age of 10–11 weeks. The animals were housed in a temperature-, humidity-, and light-controlled room with free access to tap water and standard diet. All animal experimental procedures were approved by the Animal Experiment Committee of Fujisawa Pharmaceutical Co. Ltd. (currently, Astellas Pharma Inc.).

2.3. Meal tolerance test (MTT)

A meal tolerance test was performed using conscious 12- to 13-week old rats following an overnight fast. The meal (refeeding) was then administered by allowing free access to solid food (CRF-1, Oriental Yeast, Tokyo, Japan) for 20 min (time 0–20 min). Animals that consumed less than 1 g were omitted from the experiments. ASP4000, acarbose, or the vehicle was administered orally during the first 20 min of refeeding. Blood samples was obtained by heparinized capillary pipette from tail vein following the challenge for determining the levels of plasma glucose and insulin (−30, 30, 60, and 120 min) as well as the level of GLP-1 (−30, 20, 40, 60, 90, and 120 min). In the MTT for ASP4000, animals that consumed less than 1 g and more than 4 g were omitted from the experiments. The animals were handled with care during this process in order to minimize stress.

2.4. Biochemistry determinations

Plasma glucose was measured using the Glu CII-test (Wako, Osaka, Japan). Plasma immunoreactive active GLP-1 was measured with a Glucagon-Like Peptide-1 (Active) ELISA kit (Linco Research, St. Charles, MO). Plasma immunoreactive insulin was determined with an insulin ELISA kit (Morinaga, Yokohama, Japan) using rat insulin as a standard.

2.5. Statistical analysis

Data are expressed as the mean ±S.E.M. The differences in the plasma glucose and insulin levels between the vehicle control group and drug (ASP4000 and acarbose)-treated groups were determined using Dunnett's multiple comparison test. To determine the integrated glucose and insulin response to the meal challenge, the area under the curve (AUC) of Δ plasma glucose after the meal load was calculated using the trapezoidal rule (between 0 and 2 h). The differences in the Δ plasma glucose and insulin AUC values between the vehicle- and drug (ASP4000 and acarbose)-treated groups in the mouse MTT were determined using Dunnett's multiple comparison test. The differences in the plasma Δ active GLP-1 levels between the vehicle control group and ASP4000-treated groups were determined using the Student's *t* test. Comparisons between the Zucker fatty vehicle-treated group and lean vehicle-treated group were made using the Student's *t* test. The correlation between food intake and Δ plasma glucose AUC were determined using linear regression analysis. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. New-model: the meal tolerance test

Table 1 shows food intake, Δ plasma glucose levels (time: 30 min) after the 20-min free access refeeding period, and Δplasma glucose AUC (time: between −30 min and 120 min). The food consumption for Zucker fatty rats over the 20-min period was 3.87 ± 0.22, which was greater than that of Zucker lean rats (2.51 ± 0.24 g). The Δ plasma glucose (time 30 min) and Δplasma glucose AUC were approximately twice as high as that of Zucker lean rats. No significant correlation between food intake and the Δ plasma glucose AUC was observed in Zucker lean or fatty rats (Fig. 1A and B).

3.2. Effect of plasma glucose and insulin levels due to ASP4000 administration in the MTT

Fig. 2 shows the Δ plasma glucose (Fig. 2A) and Δ plasma insulin (Fig. 2B) profile during the MTT after administration of vehicle or ASP4000. After the MTT, both Δ plasma glucose and Δ insulin levels increased in rats treated with the vehicle. These levels were significantly higher in the fatty rats than the lean rats throughout the experimental period. ASP4000's improvement of glucose tolerance was dose-related, but insulin secretion was not significantly different than that seen with the vehicle.

3.3. Effect of plasma active GLP-1 levels by ASP4000 administration in the MTT

Fig. 3 shows the plasma Δ active GLP-1 levels after administration of the vehicle or ASP4000 in the MTT. The active GLP-1 levels did not change in the vehicle-treated fatty rats after refeeding, but ASP4000 increased the active GLP-1levels constitutively.

Table 1
Food intake and plasma glucose levels in the meal tolerance test.

	Food intake (g)	Δ Plasma glucose (mg/dL, 0.5 h)	Δ Plasma glucose AUC (mg h/dL, −0.5 to 2 h)
Zucker lean rats	2.51 ± 0.24	62.4 ± 4.4	117.5 ± 7.8
Zucker fatty rats	3.87 ± 0.22	126.4 ± 7.1	223.2 ± 12.6

Data are displayed as the mean ±S.E.M. (Zucker lean rats: *n*=12, Zucker fatty rats: *n*=17). Food intake was determined after 20 min of refeeding. The Δ plasma glucose value 0.5 h after the start of meal refeeding is shown. The Δ plasma glucose AUC value between −0.5 h and 2 h in the meal tolerance test is also shown (time 0: beginning of refeeding period).