Colestimide Lowers Plasma Glucose Levels and Increases Plasma Glucagon-like Peptide-1 (7–36) Levels in Patients with Type 2 Diabetes Mellitus Complicated by Hypercholesterolemia

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Abstract

Background: Colestimide has been reported to lower blood glucose levels in patients with type 2 diabetes complicated by hypercholesterolemia.

Aim: To examine the mechanism by which colestimide decreases plasma glucose levels in the above patients.

Methods: A total of 16 inpatients with type 2 diabetes complicated by hypercholesterolemia received colestimide for 1 week after their plasma glucose levels stabilized. We measured plasma glucose, serum immunoreactive insulin (IRI), serum lipid, plasma glucagon, and plasma glucagon-like peptide-1 (GLP-1) levels. These variables at baseline and 1 week of colestimide administration were compared.

Results: Preprandial plasma glucose levels (baseline: $132 \pm 33 \text{ mg/dL}$ vs. completion: $118 \pm 43 \text{ mg/dL}$, *P*=0.073) tended to decrease after colestimide administration, while 1-hr postprandial plasma glucose levels (baseline: $208 \pm 49 \text{ mg/dL}$ vs. completion: $166 \pm 30 \text{ mg/dL}$, *P*<0.001) and 2-hr postprandial plasma glucose levels (baseline: $209 \pm 56 \text{ mg/dL}$ vs. completion: $178 \pm 39 \text{ mg/dL}$, *P*=0.015) decreased significantly at 1 week of colestimide administration. The 2-hr postprandial plasma GLP-1 level was significantly (*P*=0.015) higher at 1 week of colestimide administration as compared with the baseline level, while there were no significant changes in preprandial and 1-hr postprandial plasma GLP-1 levels.

Conclusions: The GLP-1-increasing activity of colestimide may explain, at least in part, the mechanism of its blood glucose-lowering activity in patients with type 2 diabetes complicated by hypercholesterolemia.

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Key words: colestimide, glucagon-like peptide-1, glycemic control, type 2 diabetes mellitus

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Introduction

Anion exchange resins, such as cholestyramine and colestimide, lower serum cholesterol levels by binding to bile acids in the intestinal tract. A significant improvement of glycemic control in patients with type 2 diabetes complicated by hypercholesterolemia was previously reported for cholestyramine¹. We have also observed a significant improvement of glycemic control as does acarbose in patients with type 2 diabetes treated with colestimide². Furthermore, Yamakawa et al.³ and Kawabata et al.4 recently reported that colestimide has a blood glucose-lowering effect in patients with type 2 diabetes. However, the mechanism by which an anion exchange resin decreases plasma glucose levels remains unknown. Watanabe et al. recently reported that administration of bile acids, especially cholic acids, induces energy expenditure by promoting intracellular thyroid hormone activation in brown adipose tissue, preventing obesity and insulin resistance in metabolic syndrome model mice⁵. Therefore, the involvement of an anion exchange resin in this mechanism also is conceivable.

Glucagon-like peptide-1 (GLP-1), a gastrointestinal hormone secreted by pancreatic α -cells and by Lcells in the small and large intestines that enhances insulin secretion by pancreatic β -cells⁶, is currently marketed as a hypoglycemic drug. GLP-1 secretion is closely related with the concentration of bile acids⁷. Here we report the hypoglycemic effect of colestimide by examining changes in plasma GLP-1 levels, serum insulin levels, and plasma glucagon levels at baseline and 1 week of colestimide administration.

Materials and Methods

Subjects and Design

The present study was conducted at our hospital in 16 inpatients with type 2 diabetes complicated by hypercholesterolemia (5 males and 11 females, mean age: 65.5 ± 14.4 ; BMI: $27.3 \pm 7.5 \text{ kg/m}^2$; duration of diabetes: 10.3 ± 6.1 years). Their postprandial plasma glucose levels were poorly controlled despite a weight-maintaining diet (25~30 kcal/kg of standard body weight [BW]) and treatment with oral hypoglycemic agents. Four patients were treated by alone, and diet therapy twelve with oral hypoglycemic agents. The following oral hypoglycemic agents were administered: voglibose to one patient, glibenclamide to four patients, glimepiride to four patients, gliclazide to one patient, and pioglitazone hydrochloride to two patients.

After hospital admission, self-monitoring of blood glucose (SMBG) was performed on consecutive days by all subjects. The coefficient of variance (CV) for SMBG at fasting for 1 week immediately before the first measurement of plasma glucose levels was calculated in the same way as our previous study². The above first measurement was performed after the CV value was verified to be as low as 2.9%.

Colestimide (1,500 mg) was administered orally twice daily, before breakfast and supper, for 1 week. Plasma glucose, serum insulin, plasma glucagon, and plasma GLP-1 levels were measured (preprandial, 1 hr postprandial, and 2 hr postprandial) at baseline and 1 week of colestimide administration. There were no changes in diet therapy and in doses or types of concomitant therapeutic drugs for diabetes mellitus and other drugs during this period. Each patient was given a standard meal at 8:00 after an overnight fast. The standard meal was 472 kcal with an energy distribution of 57% CHO, 24% fat, and 19% protein. BMI was calculated as weight (kg)/ height (m²). Plasma glucose levels and serum lipid levels were measured by the enzymatic method, serum HDL cholesterol levels by the dextran sulfate Mg precipitation method, serum HbA_{1c} levels by high-performance liquid chromatography (HPLC), and serum IRI levels by radioimmunoassay. Plasma GLP-1 (pmol/L) levels were measured by ELISA (EGLP-35K; Linco, St. Charles, MO). The lowest detection limit for GLP-1 by this assay is 2 pM (100 µL in plasma sample size). The intraassay CV was 9%, and the interaassay CV was 13%, with a sensitivity of 2.0 pmol/liter. For the measurement of GLP-1 amide (7-36), blood samples were put into EDTA-evacuated tubes, and 30 µL (10 µL per milliliter of blood) of a dipeptidyl peptidase IV (DPP-

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Table	1	Baseline	charact	eristics	of the	study	subjects
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Ν	16
Male/Female	5/11
Age (years old)	65.5 ± 14.4
Plasma fasting glucose (mg/dL)	132 ± 33
HbA1c (%)	8.5 ± 2.0
Duration of diabetes (years)	10.3 ± 6.1
Diet alone/oral hypoglycemic agents *	4/12
Body Mass Index (kg/m ²)	27.3 ± 7.5
TC (mg/dL)	221.6 ± 29.5
HDL-C (mg/dL)	50.8 ± 16.4
TG (mg/dL)	181.6 ± 124.0

*: One subject was treated with voglibose, four with glibenclamide, four with glimepiride, one with gliclazide and two with pioglitazone hydrochloride Data are expressed as means \pm SD.

IV) inhibitor (LC0014; Linco, St. Charles, MO) were added. The plasma, immediately obtained by centrifugation at 4° C, was stored at -80° C until assay.

Prior to the onset of the study, informed consent was obtained from all study subjects after providing a sufficient explanation to them.

Statistical analyses were conducted using paired Student's t-test. Data in the text and tables are expressed as mean \pm SD.

Plasma GLP-1 levels at baseline and 1 week of colestimide administration were examined by paired Student's t-test. Furthermore, the CV values for SMBG were calculated during 1 week before the study onset when blood glucose levels after admission stabilized relatively.

Results

Baseline characteristics of the study subjects are shown in **Table 1**.

Mean changes in plasma glucose, serum IRI, plasma glucagon, and plasma GLP-1 levels at baseline and 1 week of colestimide administration are shown in **Table 2**. Preprandial plasma glucose levels (baseline: $132 \pm 33 \text{ mg/dL}$ vs. completion: 118 \pm 43 mg/dL, *P*=0.073) tended to decrease after colestimide administration, while 1-hr postprandial plasma glucose levels (baseline: $208 \pm 49 \text{ mg/dL}$ vs.

postprandial glucose levels (baseline: 209 ± 56 mg/ dL vs. completion: $178 \pm 39 \text{ mg/dL}$, P=0.015) significantly decreased after colestimide administration. No significant difference was found between baseline and 1 week of colestimide administration with respect to serum IRI levels (preprandial, baseline: $10.1 \pm 8.5 \mu U/mL$ vs. completion: 9.9 \pm 11.6 μ U/mL, P=0.952; 1 hr postprandial, baseline: $30.9 \pm 20.6 \mu U/mL$ vs. completion: 27.4 \pm 19.9 μ U/mL, P=0.434; and 2 hr postprandial, baseline: $38.2 \pm 30.1 \mu U/mL$ vs. completion: 32.3 \pm 22.3 μ U/mL, P=0.212) and to plasma glucagon levels (preprandial, baseline: 90 ± 31 pg/mL vs. completion: 88 ± 35 pg/mL, P=0.699; 1 hr postprandial, baseline: 108 ± 38 pg/mL vs. completion: 99 \pm 43 pg/mL, P=0.139; 2 hr postprandial, baseline: 103 ± 36 pg/mL vs. completion: 96 \pm 38 pg/L, P= 0.130). The 2-hr postprandial plasma GLP-1 level was significantly higher at 1 week of colestimide administration as compared with the baseline level (baseline: 64 ± 17 pmol/L vs. completion: 72 \pm 18 pmol/L, P=0.015), while there were no significant changes in preprandial (baseline: $63 \pm 19 \text{ pmol/L}$ vs. completion: $66 \pm 20 \text{ pmol/L}$, P>0.05) and 1-hr postprandial levels (baseline: 66 \pm 15 pmol/L vs. completion: 65 \pm 22 pmol/L, P>0.05). The mean serum total cholesterol levels decreased significantly from 221.6 ± 29.5 mg/

completion: 166 \pm 30 mg/dL, P<0.001) and 2-hr

	Glucose (mg/dL)		Insulin (µU/mL)		Glucagon (pg/mL)		GLP-1 (pmol/L)	
	Before	After	Before	After	Before	After	Before	After
preprandial	132 ± 33	118 ± 43	10.1 ± 8.5	9.9 ± 11.6	90 ± 31	88 ± 36	63 ± 19	66 ± 20
1-hr postprandial	208 ± 49	166±30 **	30.9 ± 20.6	27.4 ± 19.9	108 ± 38	104 ± 46	66 ± 15	65 ± 22
2-hr postprandial	209 ± 56	178±39 *	38.2 ± 30.1	32.2 ± 22.3	103 ± 36	99 ± 40	64 ± 17	72±18 *

Table 2 Changes in plasma glucose, serum insulin, plasma glucagon, and plasma GLP-1 levels at 1 week of colestimide administration

Preprandial, and 1-hr and 2-hr postprandial values are expressed as mean \pm SD at baseline and 1 week of colestimide administration (n=16).

Data were analyzed by paired Student's t-test. * P<0.05 and ** P<0.001 for significant differences against baseline

dL to $192.1 \pm 26.8 \text{ mg/dL}$ (*P*=0.001). However, no significant difference was found in changes of serum HDL-cholesterol and serum triglyceride levels. During the study period, colestimide was not discontinued due to an adverse reaction in any patient.

Discussion

To our knowledge, our study is the first investigation which has revealed a significant increase in postprandial plasma GLP-1 levels by a significant accompanied decrease in postprandial plasma glucose levels at 1 week of colestimide administration. These results suggest that colestimide possibly decreases plasma glucose levels in patients with type 2 diabetes mellitus via the entero-insular axis. We consider that the results from this study explain, at least in part, the blood glucose-lowering effect of colestimide because the extent of increase in plasma GLP-1 levels was small. GLP-1 secretion is closely related with the concentration of bile acids⁷, and colestimide—a drug that binds to bile acids and changes the composition of bile acids in the intestine-may possibly increase plasma GLP-1 levels. GLP-1 has been postulated to correct plasma glucose levels by increasing insulin secretion (incretin action) and by inhibiting glucagon secretion. However, we found no significant differences in serum insulin and plasma glucagon levels at baseline and 1 week of colestimide administration in this study. Intervals for measurement of serum IRI and plasma glucagon levels before and after oral administration of colestimide remain undetermined. Alternatively, insulin secretion enhanced by GLP-1 may be counterbalanced by insulin sensitivity improved by colestimide. In fact, Yamakawa et al.³ revealed that colestimide administration slightly decreases HOMA-IR, an index for insulin sensitivity, in patients with type 2 diabetes. Further, cholestyramine has been reported to increase cholecystokinin (CCK)⁸, one of gastrointestinal hormones. Incretins such as CCK and glucose-dependent insulinotropic polypeptide (GIP) have also been reported to have a hypoglycemic effect^{9,10}. Further basic research is required to clarify these blood glucose-lowering mechanisms via the entero-insular axis.

Of particular interest is the lack of significant GLP-1 secretion following the ingestion of the breakfast without colestimide in patients with type 2 diabetes in the present study. Previous studies had of GLP-1 shown а significant reduction concentrations after an oral glucose $\mathsf{load}^{^{11,12,15}}$ or a mixed meal^{13-15,17} in patients with type 2 diabetes. However, in some of these previous reported studies, GLP-1 response to oral glucose^{11,12} or mixed meal^{14,16,17} did not completely abolished. DeLeon et al.18 indicated that the postprandial GLP-1 level was not significantly altered in 8 of 10 elderly patients with type 2 diabetes, although two patients showed a significant postprandial increase in serum GLP-1 levels at 1 hour after the test meal with 100 mg acarbose. The reason why the GLP-1 did not change after breakfast without colestimide administration in the present study is not clear. The secretion of GLP-

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Table 3Changes in plasma glucose, serum insulin, plasma glucagon, and plasma GLP-1 levels at baseline and at one
week after the ingestion of control meal without colestimide administration in patients with type 2 diabetes

	Glucose (mg/dL)		Insulin (µU/mL)		Glucagon (pg/mL)		GLP-1 (pmol/L)	
	Before	After	Before	After	Before	After	Before	After
preprandial	132 ± 35	131 ± 36	14.6 ± 9.9	17.6 ± 13.3	135 ± 52	128 ± 57	80 ± 14	84 ± 16
1-hr postprandial	189 ± 51	187 ± 45	35.7 ± 47.7	22.6 ± 29.8	145 ± 48	135 ± 64	82 ± 16	80 ± 14
2-hr postprandial	175 ± 62	197 ± 44	67.0 ± 49.0	72.0 ± 63.7	127 ± 49	122 ± 57	81 ± 13	80 ± 12

Preprandial, and 1-hr and 2-hr postprandial values are expressed as mean \pm SD at baseline and at one week after the ingestion of control meal without colestimide administration in patients with type 2 diabetes (n=8). Data were analyzed by paired Student's t-test.

1 is generally increased by ingestion of a meal rich in fats and complex carbohydrate in human¹⁹²⁰. Therefore more high-fat meals, like other studies¹⁶⁻¹⁸, probably may urge the increase of secretion of GLP-1 after the meal in Japanese elderly patients with type 2 diabetes Further studies will be necessary to elucidate this issue.

There are several study limitations in the present study. The interval for measurement of plasma GLP-1 levels was as long as 1 hour. Therefore, it is unknown at present whether the peak plasma level of GLP-1 is located at 2 hours after oral administration of colestimide. Toft-Nielson et al. loaded test meal within 10 to 15 minutes to measure plasma GLP-1 levels for 4 hours and reported that the peak plasma level of GLP-1 is located at 1 hour after administration and a high level is maintained also at 2 hours after administration in patients with diabetes, while a high value is maintained at 1 to 2 hours after administration in patients without diabetes¹⁴. However, the plasma half-life of GLP-1 is short, and the present study failed to reveal the precise time zone of the peak level. Therefore, we consider that a further study using a slightly shorter interval will be required.

Another limitation of this study was the absence of control. In another study in eight patients with diabetes who did not receive colestimide, we compared plasma glucose, serum IRI, plasma glucagon, and plasma GLP-1 levels at about 1 week before administration with the baseline, 1-, and 2hour postprandial values (**Table 3**). Namely, the following values were obtained: for plasma GLP-1 levels, preprandial (baseline: $80 \pm 14 \text{ pmol/L}$ vs. completion: $84 \pm 16 \text{ pmol/L}$), 1 hr postprandial (baseline: $82 \pm 16 \text{ pmol/L}$ vs. completion: 80 ± 14 pmol/L), and 2 hr postprandial (baseline: 81 ± 13 pmol/L vs. completion: 80 ± 12 pmol/L); for plasma glucose levels, preprandial (baseline: $132 \pm 35 \text{ mg/dL}$ vs. completion: $131 \pm 36 \text{ mg/dL}$), 1 hr postprandial (baseline: $189 \pm 51 \text{ mg/dL}$ vs. completion: 187 ± 45 mg/dL), and 2 hr postprandial (baseline: 175 ± 62 mg/dL vs. completion: 197 ± 44 mg/dL); for serum insulin levels, preprandial (baseline: 14.6 \pm 9.9 μ U/ mL vs. completion: 17.6 \pm 13.3 μ U/mL); 1 hr postprandial (baseline: $35.7 \pm 47.7 \mu U/mL$ vs. completion: 22.6 \pm 29.8 μ U/mL); 2 hr postprandial (baseline: $67.0 \pm 49.0 \ \mu U/mL$ vs. completion: $72.0 \pm$ 63.7 μ U/mL); and plasma glucagon levels (baseline: $135 \pm 52 \text{ pg/mL}$ vs. completion: $128 \pm 57 \text{ pg/mL}$; 1 hr postprandial (baseline: $145 \pm 48 \text{ pg/mL}$ vs. completion: $135 \pm 64 \text{ pg/mL}$; and 2 hr postprandial (baseline: $127 \pm 49 \text{ pg/mL}$ vs. completion: 122 ± 57 pg/mL). Therefore, the comparisons revealed no significant change. In conclusion, the increase in plasma GLP-1 levels is highly likely to be attributed to the oral administration of colestimide. We consider that a case-control study with a greater number of patients will be required.

Conclusions

The GLP-1-increasing activity of colestimide may explain, at least in part, the mechanism of its blood glucose-lowering activity in patients with type 2 diabetes complicated by hypercholesterolemia. The present study showed that colestimide improves not only dyslipidemia but also glycemic control in patients with type 2 diabetes complicated by hypercholesterolemia. Patients with diabetes are often predisposed to the so-called "metabolic syndrome" and are frequently complicated by hypercholesterolemia. If not only improving lipid metabolism but also having a hypoglycemic effect, colestimide is potentially beneficial for the relevant patients.

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