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The clinical course and potential underlying mechanisms of everolimus-induced hyperglycemia

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Abstract. The mechanistic target of rapamycin (mTOR) inhibitor everolimus is an antitumor agent known to cause hyperglycemia. However, the clinical course of everolimus-induced hyperglycemia, its pathophysiological basis, and the treatment strategy are not clear. In this case series report, we present the clinical course of everolimus-induced hyperglycemia in four patients. Hyperglycemia occurred 3–8 weeks after the administration of everolimus irrespective of the body mass index (range, 21.3–29.1 kg/m^2) or pre-existing diabetes. Insulin or insulin secretagogues were required for glycemic control in most of the patients. Of note, the hyperglycemia was reversible in all patients, and none of the patients required anti-diabetic agents to achieve adequate glycemic control after cessation of everolimus therapy. To investigate the underlying mechanism of everolimus-induced hyperglycemia, we assessed insulin secretion and sensitivity by 75 g oral glucose tolerance test, arginine challenge test, and/or hyperinsulinemic-euglycemic clamp study using stable isotope-labeled glucose tracer in two patients. Everolimus did not affect insulin sensitivity in the liver, skeletal muscle, or the adipose tissue. In contrast, everolimus impaired insulin secretion and thereby increased basal hepatic glucose production. These findings further our understanding of the role of mTOR in glucose homeostasis in humans and provide insights for treatment strategies against everolimus-induced hyperglycemia.

Key words: Mechanistic target of rapamycin, Everolimus, Hyperglycemia, Insulin secretion, Insulin sensitivity

THE MECHANISTIC TARGET OF RAPAMYCIN (mTOR) is a key sensor of nutritional status, which regulates growth and energy homeostasis in both normal and cancerous tissues [1]. The mTOR inhibitor everolimus is approved for the treatment of various advanced solid tumors; however, its use is associated with various metabolic side effects. Everolimus-induced hyperglycemia is one of the major side effects with a reported incidence of 12%–50% in phase III trials of everolimus [2]. The pathophysiological basis of everolimus-induced hyperglycemia is yet to be established, which has been a barrier to the development of appropriate treatment strategies. In this case series report, we describe the clinical course of everolimus-induced hyperglycemia in four patients and discuss the strategies for achievement of adequate glycemic control. In addition, we examined insulin secretion and the changes in insulin sensitivity during the course in two of these patients.

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Patients and Methods

All patients have provided written informed consent for publication of this case series report.

Arginine stimulation test

Arginine stimulation test was conducted as described previously [3]. After an overnight fast, patients were kept at rest for 30 min. Subsequently, arginine (30 g) was administered by intravenous infusion of a 10% L-arginine hydrochloride solution over 30 min. Blood was collected at preloading (0 min) and 30, 60, 90, and 120

min after arginine loading to measure serum C-peptide immunoreactivity and plasma immunoreactive glucagon. Serum C-peptide immunoreactivity was measured using a immunoenzymometric assay kit (E-test TOSOH II C-peptide II; Tosoh, Shunan, Japan) or an electrochemiluminescence immunoassay (ECLusys® C-peptide; Roche, Basel, Switzerland). Plasma immunoreactive glucagon was measured using a radioimmunoassay kit (Glucagon RIA SML; Euro-Diagnostica AB, Malmö, Sweden).

75 g oral glucose tolerance test

After an overnight fast, 75 g oral glucose tolerance test was performed at 0830 h. Blood samples were collected at 0, 30, 60 and 120 min to measure plasma glucose level and immunoreactive insulin. Immunoreactive insulin was measured using a immunoenzymometric assay kit (E-test TOSOH II IRI; Tosoh, Shunan, Japan).

Evaluation of insulin sensitivity/resistance and insulin secretion

As conventional indices for insulin sensitivity/resistance, the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity index (QUICKI) were used. The values for HOMA-IR and QUICKI were calculated using the following formulas: HOMA-IR = [fasting insulin (μU/mL) × fasting plasma glucose (mmol/L)]/22.5 and QUICKI = 1/{log [fasting plasma glucose (μU/mL)] + log[fasting insulin (mmol/L)]}.

As conventional indices for insulin secretion, the homeostasis model assessment of beta-cell function (HOMA-β) and the insulinogenic index were used. The values for HOMA-β and insulinogenic index were calculated using the following formulas: HOMA-β = {Immunoreactive insulin (IU/L) × 20/[fasting plasma glucose (mg/dL) – 63]} and insulinogenic index = [increment of plasma insulin (μU/mL) during the first 30 min of OGTT]/[increment in glucose (mg/dL) during the first 30 min of OGTT].

Hyperinsulinemic-euglycemic clamp study

Hyperinsulinemic-euglycemic clamp study was conducted as described previously [4]. After an overnight fast, patients were inserted two intravenous catheters in the antecubital vein of each arm; one for blood sampling and one for infusion of glucose, insulin, and tracers. At 0700 h, after obtaining a blood for assessing background enrichment of plasma glucose, a continuous infusion of [6,6-$^2H_2$]glucose (>99% enriched; Cambridge Isotope, Andover, MA, USA) was started at a rate of 0.05 mg·kg^{-1}·min^{-1} after a priming dose equivalent. After 100, 110, and 120 min, blood was collected to determine tracer enrichments. Subsequently, at 0900 h, the hyperinsulinemic-euglycemic clamp study was started using an artificial pancreas (model STG-55; Nikkiso, Tokyo, Japan). A primed continuous infusion of insulin (Humulin R; Eli Lilly, Indianapolis, IN, USA) was started and continued for 2.0 h at a rate of 1.25 mU·kg^{-1}·min^{-1} to attain a plasma insulin concentration of approximately 100 μU/mL. Glucose was infused to maintain a plasma glucose concentration of 100 mg/dL. Simultaneously, [6,6-$^2H_2$]glucose infusion was started and continued at a rate of 0.15 mg·kg^{-1}·min^{-1}. During the last 20 min of the clamp study, blood samples were obtained in 10-min intervals to determine tracer enrichments.

Calculating indices of organ-specific insulin sensitivity

Basal hepatic glucose production was calculated as the rate of appearance of glucose, which is calculated using Steele's equation from tracer data [4]. Hepatic glucose production during the clamp study was calculated as the difference between rate of appearance of glucose and the infusion rate of exogenous glucose. We calculated and defined organ-specific insulin sensitivity in the liver, skeletal muscle, and adipose tissue as described previously [4]. Hepatic insulin sensitivity index was calculated as insulin-induced suppression of hepatic glucose production during a clamp study. The skeletal muscle insulin sensitivity index was calculated as insulin-stimulated glucose disposal, and the adipose tissue insulin sensitivity index was calculated as insulin-induced suppression of free fatty acids during a clamp study.

Case 1

A 63-year-old man with renal cell carcinoma, who had been treated with everolimus, was referred to our division for treatment of hyperglycemia. He had a 5-month history of type 2 diabetes, which was treated with a dipeptidyl peptidase-4 (DPP-4) inhibitor, alogliptin 25 mg/day [glycosylated hemoglobin (HbA1c): 6.7%]. His father and brother had diabetes. After 5 weeks of everolimus (10 mg/day) therapy, he experienced exacerbation of hyperglycemia (HbA1c: 9.2%; casual plasma glucose: 364 mg/dL) (Fig. 1A), which necessitated intensive insulin therapy to achieve glycemic control. After switching from everolimus to axitinib or sorafenib, he achieved adequate glycemic control with metformin monotherapy (500 mg/day; HbA1c: 5.9%).

To investigate the underlying mechanism of everolimus-induced hyperglycemia, we assessed insulin secretion and organ-specific insulin sensitivity during and after the cessation of everolimus therapy. After cessation of everolimus, the arginine-stimulated C-peptide

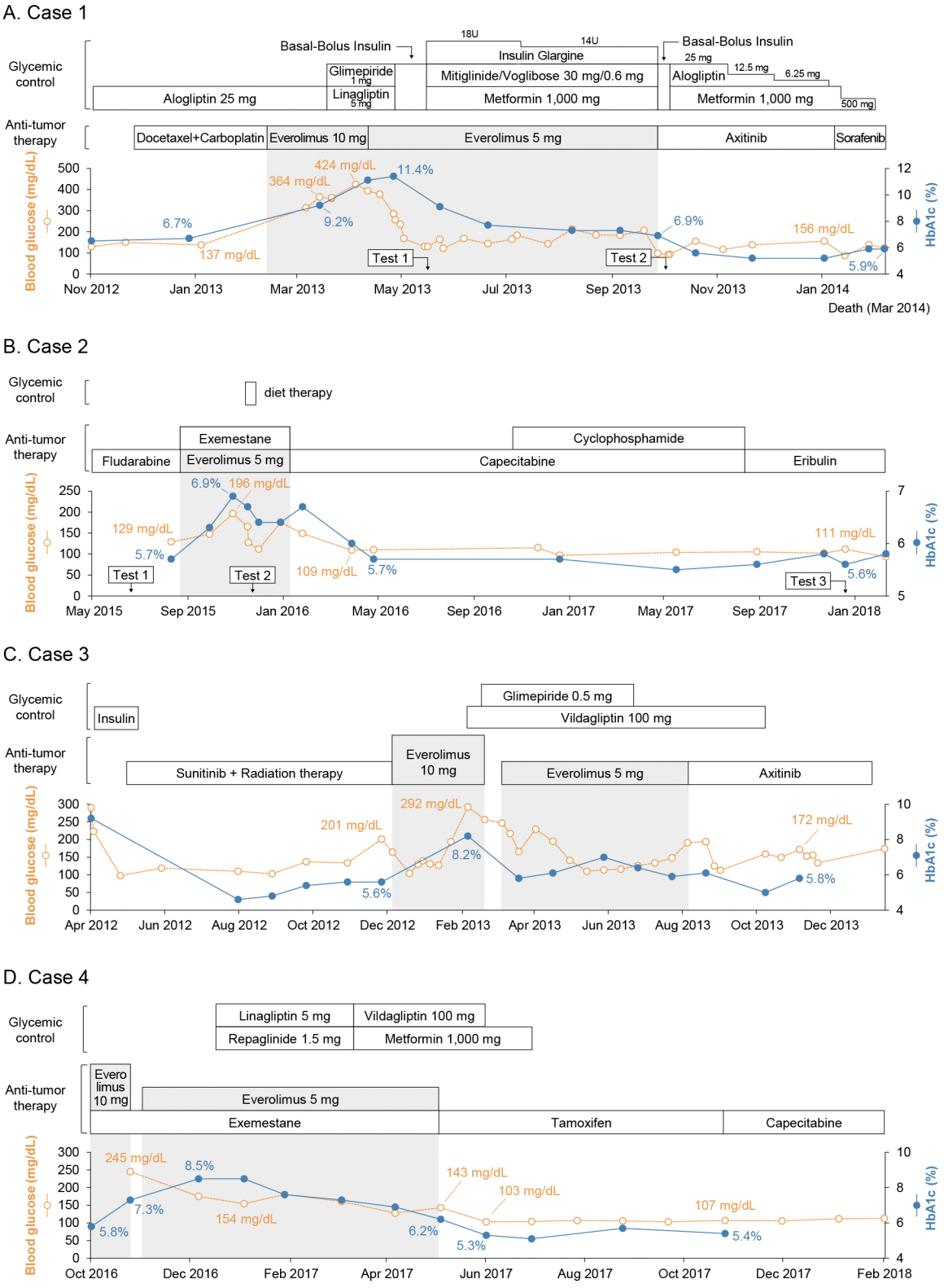

Fig. 1 Schematic illustration of the clinical course of patients with everolimus-induced hyperglycemia
HbA1c, glycosylated hemoglobin

response increased from Δ2.6 (3.2–5.8) ng/mL to Δ3.5 (3.9–7.4) ng/mL, whereas glucagon response decreased from Δ225 (139–364) ng/mL to Δ210 (118–328) ng/mL (Table 1A). In the hyperinsulinemic-euglycemic clamp study using artificial pancreas and stable isotope-labeled glucose tracer, the basal hepatic glucose production

Table 1 Insulin secretion and sensitivity before, during, and after the cessation of everolimus therapy

A. Case 1

	Everolimus	
	ON (Test 1)	OFF (Test 2)
Arginine tolerance test (0, 30, 60, 90, and 120 min)		
Blood Glucose (mg/dL)	147, 169, 174, 168, 152	95, 124, 126, 112, 101
C-peptide immunoreactivity (ng/mL)	3.2, 4.9, 5.8, 5.3, 5.1	3.9, 6.7, 7.4, 6.8, 6.3
Glucagon (pg/mL)	139, 364, 254, 181, 145	118, 328, 202, 152, 138
Hyperinsulinemic-euglycemic clamp		
Glucose infusion rate (mg/kg/min) (IIR = 1.25 mU/kg/min)	2.4	3.9
Basal hepatic glucose production (mg/kg/min)	3.59	1.47
Insulin-induced suppression of hepatic glucose production (%)	96.64	100
Insulin-stimulated glucose disposal (mg/kg/min)	2.67	2.83
Insulin-induced suppression of free fatty acids (%)	67.7	60.3

B. Case 2

	Everolimus		
	OFF (Test 1)	ON (Test 2)	OFF (Test 3)
75 g OGTT (0, 30, 60, 120 min)			
Blood Glucose (mg/dL)	106, 224, 253, 230	126, 214, 312, 297	108, 185, 227, 231, 216
Immunoreactive Insulin (μU/mL)	3.7, 30.5, —, 37.5	2.8, 12.0, 24.3, 31.2	4.3, 17.4, 35.9, 30.0, 46.2
Insulinogenic index	0.23	0.1	0.17
HOMA-β (%)	31	16	34.4
HOMA-IR	0.97	0.87	1.15
QUICKI	0.39	0.39	0.24
Arginine challenge test (0, 30, 60, 90, 120 min)			
Blood Glucose (mg/dL)	—	107, 126, 121, 104, 95	103, 126, 113, 91, 95
C-peptide immunoreactivity (ng/mL)	—	0.9, 1.6, 1.5, 1.1, 0.7	1.7, 2.7, 2.9, 2.0, 1.5
Glucagon (pg/mL)	—	102, 142, 126, 109, 107	199, 451, 252, 167, 167
Hyperinsulinemic-euglycemic clamp			
Glucose infusion rate (mg/kg/min) (IIR = 1.25 mU/kg/min)	—	3.5	3.79
Basal hepatic glucose production (mg/kg/min)	—	2.08	—
Insulin-induced suppression of hepatic glucose production (%)	—	69.4	—
Insulin-stimulated glucose disposal (mg/kg/min)	—	4.29	—
Insulin-induced suppression of free fatty acids (%)	—	85.5	—

HOMA-β, homeostasis model assesment of beta-cell function; HOMA-IR, homeostasis model assesment of insuin resistance; IIR, insulin infusion rate; OGTT, oral glucose tolerance test; QUICKI, Quantitative insulin sensitivity chek index.

decreased from 3.59 to 1.47 mg/kg/min after the cessation of everolimus therapy, and no change in the suppression of hepatic glucose production, glucose disposal, or suppression of free fatty acids was observed during the course.

Case 2

A 69-year-old woman with breast cancer, who had been treated with everolimus, was referred to our division for treatment of hyperglycemia. She had a 3 month history of type 2 diabetes and had received no treatment. Her grandfather and brother had diabetes. Her HbA1c

Table 2 Summary of patients

Patient No.	Age (years)	Sex	Pimary disease	BMI when referred to our division (kg/m^2)	Pre-diagnosed diabetes	Time by onset (weeks)	Metabolic changes under everolimus administration			
							Inslin secretion	Insulin sensitivity	Basal hepatic glucose production	Glucagon response to arginine challenge
1	63	M	Renal cell carcinoma	29.1	Yes	5	↓	→	↑	↑
2	69	F	Breast cancer	21.3	Yes	8	↓	→	—	↓
3	66	M	Renal cell carcinoma	21.3	Yes	7	—	—	—	—
4	57	F	Breast cancer	27.6	No	3	—	—	—	—

BMI, body mass index

was 5.7% before everolimus administration. After 8 weeks of everolimus (5 mg/day) therapy, she experienced exacerbation of hyperglycemia (HbA1c: 6.9%; casual plasma glucose: 196 mg/dL) (Fig. 1B). She achieved adequate glycemic control after switching from everolimus to capecitabine (HbA1c: 5.7%).

We assessed insulin secretion and sensitivity before, during, and after the cessation of everolimus therapy. The insulin secretion (as assessed by Δ30-min insulin/Δ30-min glucose during 75 g oral glucose tolerance test, arginine-stimulated C-peptide response, or HOMA-β) was decreased during everolimus therapy and recovered after cessation of everolimus. The glucagon response to arginine challenge after cessation of everolimus was higher than that during everolimus treatment. On the other hand, the insulin sensitivity/resistance indices, HOMA-IR and QUICKI, were not substantially altered during the clinical course (Table 1B).

Case 3

A 66-year-old man with renal cell carcinoma, who had been treated with everolimus, was referred to our hospital for treatment of hyperglycemia. He had a 10 month history of type 2 diabetes, which was well controlled with insulin followed by diet therapy alone (Fig. 1C). He had no family history of diabetes. After 7 weeks of everolimus (10 mg/day) therapy, he experienced exacerbation of hyperglycemia (HbA1c: 8.2%; postprandial plasma glucose: 292 mg/dL). He was started on a DPP-4 inhibitor (vildagliptin 100 mg/day) and a sulfonylurea (glimepiride 0.5 mg/day) (Fig. 1C). After reduction of everolimus dose to 5 mg/day and switching to axitinib, he achieved adequate glycemic control without any hypoglycemic drug (HbA1c: 5.8%).

Case 4

A 57-year-old woman with breast cancer, who had been treated with everolimus, was referred to our division for treatment of hyperglycemia. She had no past history or family history of diabetes. After 3 weeks of everolimus (10 mg/day) therapy, she developed hyperglycemia (HbA1c: 7.3%; casual plasma glucose: 245 mg/dL). She was started on a DPP-4 inhibitor (linagliptin 5 mg/day) and a glinide (repaglinide 1.5 mg/day) (Fig. 1D). After switching from everolimus to tamoxifen, she achieved normoglycemia with DPP-4 inhibitor (vildagliptin 100 mg/day) and metformin (1,000 mg/day) therapy (HbA1c: 5.3%). Finally, she achieved adequate glycemic control without any hypoglycemic drug (HbA1c: 5.4%).

Discussion

We described the clinical course and investigated the underlying mechanisms of everolimus-induced hyperglycemia in patients with solid tumors (Table 2). Everolimus-induced hyperglycemia occurred after 3–8 weeks of administration irrespective of the body mass index (range, 21.3–29.1 kg/m^2) or presence of diabetes. Insulin or insulin secretagogues were required for glycemic control in most of the patients. Of note, the hyperglycemia was reversible and all patients achieved adequate glycemic control after cessation of everolimus therapy without the use of any anti-diabetic agents.

The mechanism of everolimus-induced hyperglycemia in humans is yet to be elucidated. Everolimus inhibits mTORC1 and mTORC2 [5], both of which have opposite effects on insulin signaling. The mTORC1 activates S6 kinase and thereby inhibits IRS activation, which induces insulin resistance [6]; the mTORC2 directly phosphorylates Akt, which induces upregulation of insulin signaling [7, 8]. As previously reported in mice, we

initially hypothesized that everolimus induces insulin resistance mainly by inhibiting mTORC2 [7-9]. In the present study, however, everolimus did not affect insulin sensitivity in the liver, skeletal muscle, or the adipose tissue. Rather, everolimus impaired insulin secretion and thereby increased basal hepatic glucose production. This result suggests that the inhibition of mTOR in the insulin-producing pancreatic beta-cells was the main cause of glucose intolerance in the present study. Consistent with our observation, everolimus administration was earlier shown to reduce hypoglycemia in patients with insulinoma without any obvious effect on tumor size [10]. In a recent study, mTORC1 was shown to play a role in the maintenance of beta-cell function by enhancing cleavage of insulin from proinsulin [11]. Indeed, rapamycin, an mTOR inhibitor, decreases insulin secretion from mice and human islets [11]. Unfortunately, in the present study, we did not assay the proinsulin/insulin ratio, which may have confirmed this hypothesis.

In conclusion, everolimus induces reversible hyperglycemia, both in diabetic and non-diabetic patients, by impairing insulin secretion with no concomitant effect on insulin sensitivity. These findings further our understanding of the role of mTOR in glucose homeostasis in humans. Our findings provide insights that may facilitate the development of treatment strategies against everolimus-induced hyperglycemia. Since everolimus-induced hyperglycemia is manageable and reversible, we recommend prioritization of everolimus therapy if needed for cancer control with adequate glycemic control.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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