Everolimus Induces Rapid Plasma Glucose Normalization in Insulinoma Patients by Effects on Tumor As Well As Normal Tissues

HELLE-BRIT FIEBRICH,^a ESTER J.M. SIEMERINK,^a ADRIENNE H. BROUWERS,^b THERA P. LINKS,^c
WOUTER S. REMKES,^d GEKE A.P. HOSPERS,^a ELISABETH G. E. DE VRIES^a

Departments of ^aMedical Oncology, ^bNuclear Medicine and Molecular Imaging, and ^cEndocrinology, University Medical Center Groningen, Groningen, The Netherlands; ^dDepartment of Internal Medicine, Isala Klinieken, Zwolle, The Netherlands

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ABSTRACT

Background. Mammalian target of rapamycin inhibitor everolimus administered to four insulinoma patients rapidly controlled hypoglycemia (Kulke et al., *N Engl J Med* 2009;360:195–197). We wanted to identify the kinetics of everolimus effects on controlling hypoglycemia and understand underlying mechanisms.

Methods. Three consecutive patients with a metastasized symptomatic insulinoma were started on 100 µg of octreotide subcutaneously three times daily. Because of persisting hypoglycemias, treatment with daily 10 mg of oral everolimus was initiated. Serial plasma glucose levels and serum insulin levels were measured. Computer tomography (CT) scans were performed before and after 2 and 5 months of treatment. [18F]fluoro-2-deoxy-d-glucose positron emission tomography (¹⁸F-FDG-PET) scans, to visualize glucose metabolism, were made before and after 2 weeks, 5 weeks, and 5 months of treatment. The ¹⁸F-FDG uptake was quantified as the maximum standardized uptake value.

Results. All patients achieved control of hypoglycemia on everolimus within 14 days. Insulin levels were 2.5- to 6.3-fold elevated before start of treatment and declined 14%–64% after 4 weeks of treatment. CT scans showed stable disease at 2 months in all patients, with progressive disease after 5 months in one. Before treatment, both the tumor lesions and the muscles and myocardium showed high ¹⁸F-FDG uptake. Everolimus reduced tumor and muscle ¹⁸F-FDG uptake after 2 weeks by 26% ± 14% and 19% ± 41%, and after 5 months by 31% ± 13% and 27% ± 41%.

Conclusions. Everolimus normalizes plasma glucose levels in metastatic insulinoma within 14 days, coinciding with a lower glucose uptake in tumor and muscles and declining (pro)insulin levels. This effect on tumor as well as normal tissues explains the rapid controlling of hypoglycemia. *The Oncologist* 2011;16:783–787

Correspondence: E.G.E. de Vries, M.D., Ph.D., Department of Medical Oncology, University Medical Center Groningen, P.O. Box 30001, 9700 RB Groningen, The Netherlands. Telephone: +31-50-3612934; Fax: +31-50-3614862; e-mail: e.g.e.de.vries@int.umcg.nl Received July 5, 2010; accepted for publication February 22, 2011; first published online in *The Oncologist Express* on April 11, 2011. ©AlphaMed Press 1083-7159/2011/\$30.00/0 doi: 10.1634/theoncologist.2010-0222

Table 1. Characteristics of the patients, previous therapy, and biochemical response to everolimus

Patient	Sex, age (years)	Previous therapy	Insulin in mE/L/proinsulin in pmol/L (elevation above normal, x-fold)			Tumor response and progression-free survival
			Pretreatment	1 mo	5 mos	
1	M, 60		75/328 (4.4/45)	45/110 (2.7/15)	57/110 (3.4/15)	Stable disease for 8 mo(s)
2	F, 75		43/322 (2.5/44)	37/76 (2.2/10)	23/46 (1.4/6)	Stable disease for 8+ mo(s)
3	M, 55	3 cycles 5-FU + streptozocin, 6 cycles PPRT	107/254 (6.3/35)	38/110 (2.3/15)	130/115 (7.7/16)	Stable disease for 5 mo(s)

Abbreviations: F, female; 5-FU, 5-fluorouracil; M, male; PPRT, peptide receptor radionuclide therapy.

Figure 1. Plasma glucose (solid circles) and serum insulin (open squares) levels per patient. (A): Patient 1; (B) patient 2; (C) patient 3.

CASE REPORT

Expression profiling in islet cell tumors provided evidence for a role of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (mTOR) pathway, which integrates signals from growth factors and nutrients to regulate cell growth, proliferation, and survival [1]. Two recent phase II studies showed antitumor efficacy of the mTOR-inhibitor everolimus in patients with mixed islet cell tumors [2, 3]. Additionally, everolimus induced stunningly rapid control of hypoglycemias in four patients with malignant insulinomas [4]. The precise mechanism by which this was achieved is not yet unraveled. Therefore, we closely monitored glucose and (pro)insulin levels in three patients with malignant symptomatic insulinomas and visualized and quantified with positron emission tomography (PET) the glucose metabolism using [^{18}F]fluoro-2-deoxy-d-glucose as tracer (^{18}F -FDG-PET).

The patient characteristics are summarized in Table 1. All patients had severe hypoglycemic episodes despite frequent meals and octreotide; two needed continuous glucose infusions to maintain normal glucose levels. Therefore, treatment with 10 mg everolimus daily orally was initiated. In case of grade 3 toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0, the dose was temporarily reduced to 5 mg daily.

Venous plasma glucose was measured daily in the first month and weekly thereafter with an APEC glucose analyzer (APEC Inc.); serum insulin and proinsulin levels were

measured before start of treatment and monthly thereafter. Serum insulin was determined with a DSL-1600 insulin radioimmunoassay (upper level of normal 16.9 mU/L) (Beckman Coulter), and intact proinsulin (upper level of normal 7.3 pmol/L) was determined as described previously [5].

The patients underwent computer tomography (CT) scanning before start of treatment, after 2 months, and every 3 months thereafter to determine tumor response according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 [6]. ^{18}F -FDG-PET was performed at the same time points plus at 2 and 5 weeks. The ^{18}F -FDG uptake was quantified as the maximum standardized uptake value (SUVmax) in regions of interest [7]. SUVmax values were calculated for the three hottest tumor lesions, the myocardium, and the forearm muscles.

After the start of everolimus, the clinical condition improved in all patients. Before everolimus therapy during hypoglycemic episodes, insulin levels were 2.5- to 6.3-fold elevated and proinsulin levels were 35- to 45-fold elevated (Table 1). Glucose levels normalized already on day 4, 8, and 14, respectively (Fig. 1), and the glucose infusions could be discontinued. In the heavily pretreated patient, this effect subsided after 13 weeks and was temporarily restored by increasing the octreotide dose to 200 μg three times daily. Insulin levels were lower but still abnormally elevated in all after 4 weeks (Fig. 1, Table 1). This reduction further persisted in two patients but increased again in patient 3 after 5 weeks.

Figure 2. Waterfall plot showing the percentage response in size and maximum standardized uptake values, respectively, after 5 months per patient on computed tomography (white bars) and [18F]fluoro-2-deoxy-D-glucose positron emission tomography (black bars). Bars from left to right, patient 1 to 3.

Figure 4. Maximum standardized uptake values decrease on the repeated [18F]fluoro-2-deoxy-D-glucose positron emission tomography scans. Physiological uptake in the myocardium (●) and forearm (◇) is averaged for all patients, and uptake in the hottest three tumor lesions is averaged per patient. Patient 1 (■); patient 2 (▲); patient 3 (▼). Abbreviations: SUVmax, maximum standardized uptake value.

Figure 3. Patient 1. (A): [18F]fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F -FDG-PET) scans. Pathological uptake in the liver lesions and physiological uptake in the muscles and myocardium decreases during treatment with everolimus. (B): Pretreatment computed tomography (CT) scan showing a pancreatic mass and multiple liver lesions. (C): Fusion of the pretreatment CT and ^{18}F -FDG-PET confirming uptake in the pancreatic and liver lesions.

All patients experienced stable disease for, respectively, 5, 8, and 8+ months. Figure 2 shows the changes in tumor size per patient. This points to the fact that a direct antitumor effect does not require an antitumor effect in size meeting the RECIST criteria, but may well lead to reduced metabolic activity of tumor cells.

^{18}F -FDG-PET visualized the tumor lesion in the pancreas and more liver lesions than CT in all patients (Fig. 3). ^{18}F -FDG-PET before treatment showed abnormally high myocardial and muscle uptake in all patients, which largely

normalized during treatment. After 2 weeks, everolimus ^{18}F -FDG uptake in tumor and muscles decreased, with a SUVmax decrease in tumor and muscle of $26\% \pm 14\%$ (mean $\pm$ SD) and $19\% \pm 41\%$, respectively (Fig. 4). ^{18}F -FDG uptake of tumor and muscle further decreased on subsequent scans, on average $31\% \pm 13\%$ (mean $\pm$ SD) and $27\% \pm 41\%$, respectively, after 5 months.

Earlier studies with everolimus in mixed islet cell tumors reported mainly stable disease (60%–80%) and partial responses (4%–27%) [2, 3]. In insulinoma patients

Figure 5. Schematic overview illustrating the role mTOR plays in regulating cell metabolism and proliferation. From left to right: Inhibition of mTOR by everolimus reduces Akt phosphorylation and consequently GLUT transcription and translocation, thereby inducing peripheral insulin resistance. Reduced Akt phosphorylation also decreases cell proliferation, survival, and metabolism. Thirdly, everolimus directly inhibits (pro)insulin secretion by insulinoma cells.

Abbreviations: GLUT1, glucose transporter 1; IR/IGF-1, insulin receptor/insulin-like growth factor-1 receptor; IRS-1, insulin receptor substrate 1; mTORC 1/2, mammalian target of rapamycin complex 1/2; P13-K, phosphatidylinositol 3-kinase.

treated with everolimus, two experienced stable disease and two experienced a partial response [4]. The small effect on tumor size in our patients reflects these results. Other treatment options for malignant insulinoma often have a limited effect on tumor size [8–11]. Regretfully, these studies do not describe the swiftness of hypoglycemia control.

Despite the limited effect on tumor size, glucose levels normalized rapidly in our patients. First this will be due to the observed decreased (pro)insulin secretion (Fig. 5), although (pro)insulin levels remained elevated. Therefore, besides the lower (pro)insulin levels, an additional factor is responsible for glucose normalization. We saw an avid ^{18}F -FDG uptake before treatment in tumor lesions and muscles in our patients. This increased muscle uptake was also seen in euglycemic hyperinsulinemic clamping studies, in which insulin administration resulted in increased muscular uptake [12]. In our patients, everolimus induced lowering in ^{18}F -FDG tumor uptake. This was also seen in lesions of patients with non-small cell lung cancer during everolimus [13]. However, in our insulinoma patients, there was in addition a striking reduction in ^{18}F -FDG muscle uptake. This points to a general metabolic effect of everolimus. We consider downregulation of glucose transporter 1 (GLUT1) in tumor as well as normal tissues by

everolimus [14] the second reason for glucose normalization (Fig. 5). GLUT1 regulates the glucose uptake in insulin-stimulated tissues [15]. In vitro prolonged mTOR-inhibitor treatment leads to Akt-inhibition by reduced mTOR complex 2 formation [16]. As a consequence of the declined Akt-phosphorylation, transcription and translation of GLUT1 to the plasma membrane is reduced as shown in vitro for adipocytes and fibroblasts and in xenograft tumor models [14, 17]. In a xenograft pancreatic cancer model, GLUT1 reduction during mTOR inhibition coincided with a reduction in ^{18}F -FDG uptake in the xenograft [17]. GLUT1 reduction lowers glucose transport into the cells and raises plasma glucose levels, resulting in peripheral insulin resistance [18–20]. This occurs also in cancer patients and transplant patients on mTOR inhibitors [2, 20–22]. Nine percent of the neuroendocrine patients experienced plasma glucose levels above 13.9 mmol/L [2]; in renal transplant patients that receive lower everolimus doses, this was 5.9% [23]. However, even in renal transplant patients treated with the mTOR inhibitor sirolimus, the oral glucose tolerance test showed already a 30% increase in incidence of impaired glucose tolerance [20].

In conclusion, the effect of everolimus on glycemic control in metastatic insulinoma patients is the consequence of a de-

crease of (pro)insulin levels and anatomical anti-tumor effects as well as a the consequence of peripheral insulin resistance, pointing to both on- and off-target effects of everolimus.

AUTHOR CONTRIBUTIONS

Conception/Design: Helle-Brit Fiebrich, Ester J. M. Siemerink, Elisabeth G. E. de Vries

Provision of study material or patients: Helle-Brit Fiebrich, Ester J. M. Siemerink, Adrienne H. Brouwers, Wouter S. Remkes, Geke A. P. Hospers
Collection and/or assembly of data: Helle-Brit Fiebrich, Ester J. M. Siemerink, Adrienne H. Brouwers

Data analysis and interpretation: Helle-Brit Fiebrich, Adrienne H. Brouwers, Thera P. Links, Elisabeth G. E. de Vries

Manuscript writing: Helle-Brit Fiebrich, Ester J. M. Siemerink, Adrienne H. Brouwers, Thera P. Links, Geke A. P. Hospers, Elisabeth G. E. de Vries

Final approval of manuscript: Helle-Brit Fiebrich, Ester J. M. Siemerink, Adrienne H. Brouwers, Thera P. Links, Wouter S. Remkes, Geke A. P. Hospers, Elisabeth G. E. de Vries

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