The early effect of sunitinib on insulin clearance in patients with metastatic renal cell carcinoma

Anna Maria J. Thijs,^{1,2} Cees J. Tack,³ Winette T. A. van der Graaf,^{2,4}
Gerard A. Rongen¹ & Carla M. L. van Herpen²

¹Department of Pharmacology-Toxicology, Radboudumc, Nijmegen, ²Department of Medical Oncology, Radboudumc, Nijmegen, ³Department of Internal Medicine, Radboudumc, Nijmegen, The Netherlands, ⁴The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust, London, UK

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Hypoglycaemia has been reported as side effect of sunitinib.

WHAT THIS STUDY ADDS

- This study demonstrates that sunitinib inhibits insulin clearance.
- Sunitinib could induce hypoglycaemias in patients using insulin or insulin-secretion stimulating agents.

Correspondence

Dr Anna Maria J. Thijs, MD, Department of Medical Oncology 452, Radboud University Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands.
Tel.: +31 24 361 7449
Fax: +31 24 354 0788
E-mail: annemarie.thijs@hotmail.com

Keywords

diabetes, hypoglycaemia, insulin, renal cell carcinoma, sunitinib, tyrosine kinase inhibitor

Received

23 July 2015

Accepted

1 October 2015

**Accepted Article
Published Online**

8 October 2015

AIMS

In patients with diabetes treated with sunitinib symptomatic hypoglycaemia has been reported. To explore the mechanism of this adverse effect we performed a prospective study to investigate the effect of sunitinib on insulin concentration, insulin clearance and insulin sensitivity.

METHODS

We studied the early effects of sunitinib on insulin sensitivity and insulin clearance with a hyperinsulinaemic euglycaemic clamp (insulin infusion rate $60 \text{ mU m}^{-2} \text{ min}^{-1}$; steady-state 90–120 min) in patients with renal cell carcinoma before and 1 week after the start of sunitinib 50 mg day^{-1} . Insulin sensitivity index (S_i) was defined as steady-state glucose disposal divided by the steady-state plasma insulin.

RESULTS

Ten patients (one with diabetes, treated with metformin) were included in the study protocol. Steady-state insulin concentrations during the clamp increased after 1 week of sunitinib (from $128.9 \pm 9.0 \text{ mU l}^{-1}$ to $170.8 \pm 12.8 \text{ mU l}^{-1}$, $P < 0.05$; 95% CI on difference = 64.3, –19.6). The calculated insulin sensitivity index decreased from 0.22 ± 0.04 before to $0.18 \pm 0.02 \text{ } \mu\text{mol kg}^{-1} \text{ min}^{-1} \text{ per mU l}^{-1} \text{ insulin}$ ($P < 0.05$; 95% CI on difference 0.07, 0.08). As the insulin infusion rate was similar for both clamps, the increased steady-state insulin concentration indicates reduced insulin clearance.

CONCLUSION

Sunitinib affects insulin clearance which could possibly lead to overexposure to insulin in patients using insulin or insulin-secretion stimulating agents.

Introduction

The development of tyrosine kinase inhibitors (TKIs) targeting the vascular endothelial growth factor (VEGF) has improved cancer treatment. However their use can be hampered by side effects. Sunitinib, a VEGFR-TKI, is currently used for the treatment of metastatic renal cell carcinoma (mRCC), gastrointestinal stromal cell tumours (GIST) and pancreatic neuroendocrine tumours.

The most reported side effects of sunitinib are hypertension, fatigue, hand-foot syndrome and diarrhoea [1, 2]. Several case reports and retrospective studies mention the occurrence of hypoglycaemia during treatment with sunitinib in patients with diabetes mellitus treated with a sulfonylurea derivate or insulin [3–6]. To explore the mechanism of this adverse effect we performed a prospective study to investigate the effect of sunitinib on insulin concentration, insulin clearance and insulin sensitivity.

Methods

After approval of the protocol (NCT01227213) by the Institutional Review Board of the Radboud University Medical Center, a total of 10 mRCC patients with an indication to start sunitinib were recruited. All participants provided written informed consent. Patients were eligible if they had a life expectancy of more than 12 weeks, a WHO performance status of 0–2 and no evidence of severe or uncontrolled diseases other than renal cell carcinoma. Patients treated with corticosteroids or oral anti-coagulants were excluded. All studies were performed at the Radboud University Medical Center according to institutional and Good Clinical Practice guidelines. In the week before and 1 week (7–10 days) after starting treatment with sunitinib (50 mg day⁻¹), a 120 min hyperinsulinaemic euglycaemic clamp was performed.

The experiments were performed in the morning after an overnight fast. Patients took their medication in the morning of the experiment with a cup of water. Subjects were studied in the supine position in a temperature-controlled room (23–24 °C). Before the start of the experiment blood samples were obtained for the measurement of fasting insulin and glucose. Two intravenous cannulae were inserted. One was positioned retrogradely into a dorsal vein of the hand that was placed in a plexiglass box, ventilated with heated air, for sampling of arterialized venous blood [7]. The second cannula was inserted in an antecubital vein of the contralateral arm for infusion of insulin and glucose. During the clamp insulin (Insulin aspart; Novorapid®; NovoNordisk, Bagsvaerd, Denmark; diluted in NaCl 0.9% to a concentration of 1 U ml⁻¹, with the addition of 2 ml whole blood per 50 ml) was infused at a rate of

60 mU min⁻¹m⁻² body surface area. Arterialized venous plasma glucose determinations were performed at 5 min intervals using an enzymatic-amperometric method (Biosen C-line GP+; EKF-diagnostic GmbH, Barleben, Germany). Plasma glucose was clamped at glucose 5.0 mmol l⁻¹ (i.e. 90 mg dl⁻¹), to reach an euglycaemic state, by a variable infusion of glucose 20% solution. The validity of the glucose clamp measurements of insulin sensitivity depends on achieving steady-state conditions. The *t*_{1/2} of insulin in plasma is 4–6 min, so after 30 min insulin levels have already reached steady-state. However glucose concentration and infusion should also reach steady-state with a coefficient of variation of <5%. Therefore the period of 90–120 min of the clamp is as steady-state. At 90 and 120 min after the start of the clamp, venous blood was sampled and after centrifugation the supernatant was stored at –80 °C until the measurement of insulin in all samples of all patients by radio-immunoassay at the same time. Plasma insulin was assessed by an in-house radioimmunoassay (RIA) using ¹²⁵I-labelled human insulin and anti-human insulin antiserum raised in guinea pig. Bound and free tracer were separated by sheep anti-guinea pig antiserum and precipitation by means of polyethylene glycol (PEG). The interassay coefficient of variation (CV) for the insulin measurement was 4.7% and the intra-assay coefficient of variation was 9.7% at a level of 34 mU l⁻¹.

Statistical analysis

For calculation of the whole body glucose disposal (M value) during the euglycaemic clamp the following formula was used:

$$\begin{aligned} \text{M value} = & \frac{\text{Mean glucose infusion 90–120 min (mg min}^{-1}\text{)}}{\text{Weight (kg)}} \\ & \times \frac{1000}{180 \text{ g mol}^{-1}} \text{ } \mu\text{mol kg}^{-1} \text{ min}^{-1} \end{aligned}$$

The insulin sensitivity index [$\mu\text{mol kg}^{-1} \text{ min}^{-1}$ per mU l⁻¹] is a measure of insulin sensitivity in relation to the plasma insulin concentration during the steady-state of the euglycaemic clamp [8]. The insulin sensitivity index was calculated by dividing the M value by the mean insulin concentration during the euglycaemic clamp.

Differences in parameters before and after the start of sunitinib were statistically analyzed using a parametric Student's *t*-test for paired observations. Data are presented as mean $\pm$ SEM. A value of *P* < 0.05 was considered to be statistically significant.

Results

Baseline characteristics

Between March 2012 and January 2013 10 patients signed informed consent and were included in the study. All patients had mRCC and sunitinib was the choice of treatment as decided by their oncologist. Mean age was 59 (range 43–72) years. One patient had diabetes and used metformin. Sunitinib treatment for 1 week had no significant effect on fasting glucose, fasting insulin, BMI or blood pressure (Table 1). Creatinine increased significantly after the start of sunitinib. However several studies show that the pharmacokinetics of insulin aspart are not affected by impaired renal function [9, 10].

Insulin sensitivity

During the hyperinsulinaemic euglycaemic clamp, glucose concentrations were stable but mean concentrations were not completely similar (before $4.84 \pm 0.10 \text{ mmol l}^{-1}$ and after 1 week of sunitinib $5.06 \pm 0.09 \text{ mmol l}^{-1}$, $P < 0.05$). The mean coefficient of variation for blood glucose during steady-state was $<5\%$ in both experiments. Sunitinib treatment did not increase whole body glucose disposal (M value) during the clamp ($26.5 \pm 3.7 \mu\text{mol kg}^{-1} \text{ min}^{-1}$ before start vs. $28.5 \pm 3.1 \mu\text{mol kg}^{-1} \text{ min}^{-1}$, $P > 0.05$).

Mean plasma insulin concentrations (Table 1) obtained during the steady-state of the clamp were significantly higher after 1 week of sunitinib treatment ($128.9 \pm 9.0 \text{ mU l}^{-1}$ vs. $170.8 \pm 12.8 \text{ mU l}^{-1}$; $P < 0.02$) (95% CI on difference $-64.3, -19.6$) (Figure 1), indicating reduced clearance of insulin as insulin was infused at a fixed rate.

Before start of sunitinib, the patients were insulin resistant compared with previously studied lean healthy

Figure 1

Effect of sunitinib on individual insulin concentrations during steady-state (90–120 min) of the hyperinsulinaemic clamp before (baseline) and 1 week after the start of sunitinib treatment. * $P < 0.02$.

volunteers [11], as shown by a calculated insulin sensitivity index of $0.22 \pm 0.04 \mu\text{mol kg}^{-1} \text{ min}^{-1} \text{ per mU l}^{-1}$ (95% CI on difference 0.07, 0.08). After 1 week of sunitinib, the calculated insulin sensitivity index decreased to $0.18 \pm 0.02 \mu\text{mol kg}^{-1} \text{ min}^{-1} \text{ per mU l}^{-1}$ ($P < 0.05$) suggesting a decrease in insulin sensitivity.

Discussion

The present study demonstrates for the first time that sunitinib reduces insulin clearance in patients with mRCC. This conclusion is based on the higher steady-state insulin concentrations during the clamp, where insulin is infused in a fixed infusion rate when subjects were treated with sunitinib.

Table 1

Baseline and clamp characteristics

Baseline characteristics (n = 10)		
Age (mean + range) (years)	59 (43–72)	
Gender (male/female)	9/1	
BMI ($\pm$ SE)	27.2 ± 1.1	
Diabetes (n)	1 (uses metformin)	
	Before start sunitinib	After 1 week of sunitinib
Systolic blood pressure (mmHg)	134.0 ± 3.8	140.5 ± 3.1
Diastolic blood pressure (mmHg)	80.4 ± 3.5	84.2 ± 3.7
Glucose (fasting) (mmol l^{-1})	5.1 ± 0.2	5.0 ± 0.2
Insulin (fasting) (mU l^{-1})	13.8 ± 1.3	14.1 ± 2.7
Creatinine (mmol l^{-1})	104.5 ± 10.0	$122.2 \pm 12.6^*$
Hyperinsulinaemic euglycaemic clamp	Before start sunitinib	After 1 week of sunitinib
Insulin (mU l^{-1}) at 90 min	127.0 ± 10.2	$176.9 \pm 13.7^*$
Insulin (mU l^{-1}) at 120 min	130.8 ± 8.4	$164.7 \pm 14.7^*$
Mean insulin (mU l^{-1})	128.9 ± 9.0	$170.8 \pm 12.8^*$

All data presented as mean $\pm$ SE; * $P < 0.05$ compared with before start of sunitinib. Conversion of glucose: $\text{mmol l}^{-1} \times 18.02 = \text{mg dl}^{-1}$

The mechanism of the reduced insulin clearance during sunitinib treatment is not yet known. Insulin clearance is a complex phenomenon and depends on metabolic degradation, which is thought to be largely receptor mediated [12, 13]. The liver is the primary site for insulin clearance, removing 40–80% during the first portal passage [13]. The first step in insulin clearance is binding to its receptor on the cell membrane activating the tyrosine kinase pathway leading to internalization of insulin followed by degradation in lysosomes [14–17]. *In vitro* studies have indicated that insulin internalization and insulin action are linked [18]. Thus insulin-induced insulin receptor activation not only mediates insulin actions but is also involved in its clearance. Sunitinib has shown binding affinity with the insulin receptor and inhibits the insulin-like growth factor type 1 receptor inducing ubiquitination and thereby degradation of this receptor [19, 20]. Therefore, it is likely that sunitinib can inhibit the kinase activity of the insulin receptor and consequently hamper insulin internalization and degradation.

Besides the effect on insulin clearance we observed a significant decrease in the calculated insulin sensitivity index, suggesting a decline in insulin sensitivity. However comparisons of insulin sensitivity during hyperinsulinaemic euglycaemic clamps are only valid if the same conditions are reached for all patients [21]. In our subjects the same insulin infusion rate was used throughout the experiment, however insulin concentrations were significantly higher after the start of sunitinib and moreover glucose clamp levels differed significantly. Therefore we cannot draw firm conclusions from these measurements regarding insulin sensitivity. It is intriguing that the potential effect of sunitinib on insulin sensitivity is congruent with its effect on insulin clearance, supporting the notion that both effects are mediated by the interaction of sunitinib with the insulin receptor.

Some limitations to our study should be addressed. First, we did not observe an effect of sunitinib on fasting blood glucose and fasting insulin. However in our population of patients not treated with insulin or sulfonylurea derivatives, normal regulation of endogenous insulin secretion must have compensated for the reduced insulin clearance by reduced pancreatic release of insulin. A similar study in patients with diabetes would be interesting to confirm the effect of sunitinib on insulin clearance in this population. However this is not feasible considering the size of the patient population and the sample size needed. Second, C-peptide concentration, as a measure of endogenous insulin production, was not measured. Under clamp conditions, C-peptide concentrations as marker of endogenous insulin production are typically suppressed [22, 23]. Third, creatinine increased significantly after the start of sunitinib. However several studies have shown that the pharmacokinetics of insulin aspart are not affected by this level

of impaired renal function [9, 10]. Fourth given the need to start active and standard treatment in patients with progressive metastatic disease, it was not deemed proportional to perform a placebo controlled study or to delay the start of treatment to exclude a sequence/time effect with the first aim of understanding the cause of reported hypoglycaemia.

In summary, treatment for 1 week with sunitinib reduced insulin clearance in patients with mRCC. In patients using insulin or insulin-secretion stimulating agents the effect of sunitinib on insulin clearance could in theory result in overexposure to insulin and thereby induce hypoglycaemia. Further research into the effect of sunitinib on insulin levels in clinical practice should be performed to see whether the observed effect is of clinical relevance to patients.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare AT had financial support by a personal grant from the Dutch Cancer Society and The Netherlands Foundation of Cardiovascular Excellence for the submitted work, there are no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

This study was presented as a poster at the European Society of Medical Oncology meeting in 2014.

REFERENCES

- 1 Bhojani N, Jeldres C, Patard JJ, Perrotte P, Suardi N, Hutterer G, Patenaude F, Oudard S, Karakiewicz PI. Toxicities associated with the administration of sorafenib, sunitinib, and temsirolimus and their management in patients with metastatic renal cell carcinoma. *Eur Urol* 2008; 53: 917–30.
- 2 Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; 356: 115–24.
- 3 Demirci A, Bal O, Durnali A, Ekinci AS, Esbah O, Alkis N, Oksuzoglu B. Sunitinib-induced severe hypoglycemia in a diabetic patient. *J Oncol Pharm Pract* 2013; 20: 469–72.
- 4 Templeton A, Brandle M, Cerny T, Gillissen S. Remission of diabetes while on sunitinib treatment for renal cell carcinoma. *Ann Oncol* 2008; 19: 824–5.
- 5 Billemont B, Medioni J, Taillade L, Helley D, Meric JB, Rixe O, Oudard S. Blood glucose levels in patients with metastatic

- renal cell carcinoma treated with sunitinib. *Br J Cancer* 2008; 99: 1380–2.
- 6** Agostino NM, Chinchilli VM, Lynch CJ, Koszyk-Szewczyk A, Gingrich R, Sivik J, Drabick JJ. Effect of the tyrosine kinase inhibitors (sunitinib, sorafenib, dasatinib, and imatinib) on blood glucose levels in diabetic and nondiabetic patients in general clinical practice. *J Oncol Pharm Pract* 2011; 17: 197–202.
- 7** Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metab Clin Exp* 1981; 30: 936–40.
- 8** DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; 237: E214–23.
- 9** Holmes G, Galitz L, Hu P, Lyness W. Pharmacokinetics of insulin aspart in obesity, renal impairment or hepatic impairment. *Br J Clin Pharmacol* 2005; 60: 469–76.
- 10** Kulozik F, Hasslacher C. Insulin requirements in patients with diabetes and declining kidney function: differences between insulin analogues and human insulin? *Ther Adv Endocrinol Metab* 2013; 4: 113–21.
- 11** Tack CJ, Ong MK, Lutterman JA, Smits P. Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance. Effects of troglitazone. *Diabetologia* 1998; 41: 569–76.
- 12** Duckworth WC. Insulin degradation: mechanisms, products, and significance. *Endocr Rev* 1988; 9: 319–45.
- 13** Duckworth WC, Hamel FG, Peavy DE. Hepatic metabolism of insulin. *Am J Med* 1988; 85: 71–6.
- 14** Roth RA, Cassell DJ. Insulin receptor: evidence that it is a protein kinase. *Science* 1983; 219: 299–301.
- 15** Hari J, Roth RA. Defective internalization of insulin and its receptor in cells expressing mutated insulin receptors lacking kinase activity. *J Biol Chem* 1987; 262: 15341–4.
- 16** Carpentier JL, Paccaud JP, Backer J, Gilbert A, Orci L, Kahn CR, Baecker J. Two steps of insulin receptor internalization depend on different domains of the beta-subunit. *J Cell Biol* 1993; 122: 1243–52.
- 17** Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. *Endocr Rev* 1998; 19: 608–24.
- 18** Jochen AL, Berhanu P. Insulin-stimulated glucose transport and insulin internalization share a common postbinding step in adipocytes. *Diabetes* 1987; 36: 542–5.
- 19** Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT, Faraoni R, Floyd M, Hunt JP, Lockhart DJ, Milanov ZV, Morrison MJ, Pallares G, Patel HK, Pritchard S, Wodicka LM, Zarrinkar PP. A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol* 2008; 26: 127–32.
- 20** Shen H, Fang Y, Dong W, Mu X, Liu Q, Du J. IGF-1 receptor is down-regulated by sunitinib induces MDM2-dependent ubiquitination. *FEBS Open Bio* 2012; 2: 1–5.
- 21** Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endoc Metab* 2008; 294: E15–26.
- 22** Kim CH, Park JY, Shong YK, Hong SK, Kim GS, Lee KU. Suppression of endogenous insulin secretion by exogenous insulin in patients with insulinoma. *Clin Endocrinol* 2000; 52: 87–92.
- 23** Nauck MA, Baum F, Seidensticker F, Roder M, Dinesen B, Creutzfeldt W. A hyperinsulinaemic, sequentially eu- and hypoglycaemic clamp test to characterize autonomous insulin secretion in patients with insulinoma. *Eur J Clin Invest* 1997; 27: 109–15.