Mechanism of impaired glucose metabolism during nilotinib therapy in patients with chronic myelogenous leukemia

Nilotinib, a 2nd generation tyrosin kinase inhibitor (TKI), is efficacious in the treatment of chronic myelogenous leukemia (CML).[1] Impaired glucose metabolism with hyperglycemia represents one of the most frequently observed adverse events (AEs) during nilotinib therapy.[1] However, the mechanism of glucose metabolism impairment remains unknown.

We, therefore, conducted a study with the aim of identifying the possible mechanism of glucose metabolism impairment under nilotinib therapy: i) impaired insulin secretion (including the possible role of a nilotinib-induced perturbation of incretin secretion); or ii) development of insulin resistance (including the possible role of nilotinib-induced adipokine alterations). Our analysis included 10 patients with CML without a medical history of diabetes mellitus (DM) who had newly initiated nilotinib therapy. Prior to and after three months of nilotinib treatment, all patients received a physical examination, blood count, and fasting biochemical analysis, including glucose, insulin, C-peptide, glycosylated hemoglobin (HbA1C), serum lipids, incretins (glucagon like polypeptide-1, GLP-1), glucose-dependent insulinotropic peptide (GIP)], adipokines (adiponectin, serum adipocyte fatty acid-binding protein, FABP), and trough nilotinib plasma concentration (C_{trough}). A 75 g oral glucose tolerance test (OGTT) was performed at both time points. Fasting, 1-h and 2-h glucose, insulin, C-peptide, and incretins were assessed during the OGTT. Established models were used to estimate insulin sensitivity/resistance (HOMA2-IR, HOMA2-%S, and $ISI_{0,120}$) and B-cell function (HOMA2-%B).[2,3]

Ten patients with a median age of 57 years (range 26-74) were included in our study. Four of these 10 patients received nilotinib as first line, and 6 of 10 received the drug as a second or subsequent line CML therapy. Five of 10 patients received 600 mg and 5 of 10 received 800 mg of nilotinib/d. Patients' metabolic characteristics at baseline and after three months of nilotinib therapy are presented in Table 1. The median nilotinib C_{trough} was 1510 (range 411-2973) ng/mL.

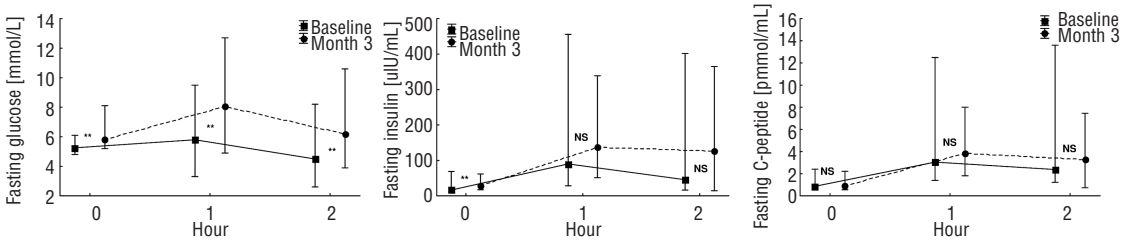
Fasting, 1-h and 2-h plasma glucose concentrations obtained during the OGTT significantly increased after three months of nilotinib therapy (Table 2, Figure 1). Two patients fulfilled criteria of diabetes mellitus (DM) during nilotinib treatment based on fasting glucose (8.1 and 7.3 mmol/L, respectively), and 2 additional patients displayed impaired glucose tolerance based on the OGTT (2-h plasma glucose concentration of 8.4 and 9.1 mmol/L, respectively).

Nilotinib administration also significantly increased fasting insulinemia (Table 2). Moreover, there was also a trend of higher postprandial insulinemia obtained during OGTT at the 3rd month of treatment (Figure 1).

There was no significant change in C-peptide concentrations (fasting and during OGTT) and fasting HbA1C after the initiation of nilotinib therapy (Table 2, Figure 1).

The euglycemic clamp represents the standard method for estimating insulin sensitivity and β-cell function. However, it is impractical for daily clinical practice, and therefore mathematical models highly correlated with the euglycemic clamp, such as the HOMA2 or $ISI_{0,120}$, are commonly used.[4] In our study, insulin resistance, as calculated by HOMA2-IR, significantly increased during nilotinib therapy (P=0.008). Moreover, there was a significant decrease in the derived insulin sensitivity index HOMA2-%S and additional ISI0,120 (P=0.006 and P=0.002, respectively; *Online Supplementary Figure S1*). While HOMA2-IR and HOMA2-%S are surrogate markers of insulin sensitivity at the basal state and tend to represent hepatic insulin resistance, $ISI_{0,120}$ reflects peripheral (mostly muscle) insulin resistance. Thus, together with marked hyperinsulinemia, we found global insulin resistance develops promptly after the initiation of nilotinib treatment.

B-cell function, as calculated by HOMA2-B%, as well as the secretion of incretins (GLP-1 and GIP), which are postprandial enhancers of insulin production,[5] were not affected by nilotinib administration (P=0.922, P=0.106, and P=0.922, respectively) (Table 2, *Online Supplementary Figure S1*). However, when we compared B-cell function at the start and after three months of nilotinib therapy (calculated as HOMA2-B%3 moths – HOMA2-B%start), we



	Fasting			1 hour			2 hours		
	Start	Month 3	p	Start	Month 3	p	Start	Month 3	p
Glucose [mmol/l] Median (range)	5.25 (4.8 - 6.1)	5.80 (5.2 - 8.1)	**0.009**	5.80 (3.3 - 9.5)	8.05 (4.9 - 12.7)	**0.027**	4.50 (2.6 - 8.2)	6.20 (3.9 - 10.6)	**0.027**
Insulin [µIU/ml] Median (range)	16.6 (10.8 - 69.0)	27.8 (17.2 - 61.9)	**0.049**	90.3 (28.6 - 455.5)	136.2 (51.3 – 339.0)	0.322	45.7 (16.6 – 402.0)	127.0 (14.7 - 364.8)	0.193
C-peptide [pmol/ml] Median (range)	0.87 (0.54 - 2.40)	0.94 (0.55 - 2.21)	0.275	3.05 (1.40 - 12.49)	3.82 (1.82 - 8.01)	0.193	2.39 (1.22 - 13.60)	3.28 (0.73 - 7.46)	0.232

Figure 1. Dynamic parameters obtained during the oral glucose tolerance test (OGTT). Data are presented as median (range). **Statistically significant at a 5% level of significance; NS: not significant.

Table 1. Patients' metabolic characteristics at baseline and after three months of nilotinib therapy.

	Median (range)/n
Baseline BMI (kg/m²)	27.2 (23.4-32.7)
3rd month BMI (kg/m²)	26.4 (23.2-32.7)
Baseline waist circumference (cm)	93.5 (77.0-108.0)
3rd month waist circumference (cm)	91.0 (78.0-104.0)
Diabetes mellitus type 2 in medical history	0
Diabetes mellitus type 2 in family history	3
Impaired glucose metabolism at baseline – impaired fasting glucose/impaired glucose tolerance/diabetes mellitus	3/0/0
Impaired glucose metabolism at the 3rd month – impaired fasting glucose/impaired glucose tolerance/diabetes mellitus	0/2/2
Hypertension in medical history	5
Dyslipidemia in medical history	1
Total serum cholesterol > 5.0 mmol/L at baseline	3
Total serum cholesterol > 5.0 mmol/L at the 3rd month	5

found a significant decrease in B-cell function in the 2 DM patients compared to the 8 other non-DM patients (P=0.024) (*Online Supplementary Figure S2*).

Based on our finding, we assume that nilotinib does not directly affect B cells. However, an organism must compensate for nilotinib-induced tissue insulin resistance through compensatory hyperinsulinemia to maintain euglycemia. In individuals with predisposed impaired B-cell function, a sudden insulin requirement could lead to prompt B-cell exhaustion. This subsequently manifests as glucose metabolism impairment, which is a reported AE of nilotinib therapy.

The mechanism of insulin resistance under nilotinib exposure at the cellular level likely occurs on the postreceptor level.[6] *In vitro* results indicate that c-ABL is involved in the insulin receptor (IR) signaling pathway. Upon insulin stimulation, c-ABL enhances an IR-dependent metabolic effect while attenuating the mitogenic effect by inducing a decrease in FAK phosphorylation, resulting in decreased ERK and increased AKT activity.[7,8] Thus, it is hypothesized that blocking c-ABL during TKI therapy affects the IR metabolic pathway.[6] However, clinically evident differences in the influence of the IR pathway among individual TKIs have not yet been studied.

In addition, results from our adipokine analyses suggest postreceptor insulin resistance. Circulating FABP9 levels did not change during nilotinib treatment. However, the plasma concentration of adiponectin, which exerts a potent insulin-sensitizing effect,[10] decreased (Table 2). This corresponds to published data reporting hyperinsulinemia subsequently causing significant hypoadiponectemia under euglycemic conditions.[11]

Taken together, our findings also have other important implications. Hyperinsulinemia,[12] postreceptor insulin resistance,[13] and hypoadiponectemia[14] play critical roles in the development of dyslipidemia and atherosclerosis. Thus, our results not only explain the rapid and significant increase in total and non-HDL cholesterol levels observed in this study (Table 2), but also a possible mechanism of the development of peripheral artery occlusion (PAO), which was recently described as an important AE of nilotinib therapy.[15]

In summary, for the first time we have clarified the mechanism of impaired glucose metabolism in CML patients treated with nilotinib, which occurs via rapidly developed tissue insulin resistance and compensatory hyperinsulinemia. It also at least partially explains the fast development of dyslipidemia and probably also PAO in these CML patients. However, further studies that include a larger patient cohort, as well as *in vitro* tests to confirm postreceptor insulin resistance, are needed, and these are already ongoing in our study group.

Table 2. Glucose and lipid metabolism assessed at the start and after three months of nilotinib therapy.

	Start median (range)	Month 3 median (range)	P
Fasting glucose (mmol/L)	5.25 (4.8-6.1)	5.8 (5.2-12.7)	0.009
Fasting insulin (µIU/mL)	16.6 (10.8-69.0)	27.8 (17.2-61.9)	0.049
Fasting C-peptide (pmol/mL)	0.87 (0.54-2.40)	0.94 (0.55-2.21)	0.275
Fasting HbA1c (%)	3.80 (3.4-4.2)	3.9 (3.2-4.7)	0.725
Incretins			
Fasting GLP-1(pM)	5.4 (4.7-6.5)	5.3 (4.6-6.4)	0.106
2-h stimulated GLP-1 (pM)	6.3 (4.7-10.5)	6.4 (4.5-10.5)	0.160
Fasting GIP (pg/mL)	49.5 (22.9-120.5)	58.5 (14.6-104.2	0.922
2-h stimulated GIP (pg/mL)	236.3 (47.2-332.2)	150.2 (73.8-270.8)	0.232
Adipokines			
Fasting FABP (ng/mL)	15.7 (5.3-37.5)	17.3 (5.6-55.3)	0.241
Fasting adiponectin (mg/L)	13.8 (0.7-45.1)	7.8 (1.0-22.3)	0.027
Serum lipids			
Total cholesterol (mmol/L)	4.75 (3.5-6.6)	5.35 (4.5-7.0)	0.013
Triglycerides (mmol/L)	1.03 (0.58-2.8)	1.29 (0.6-3.0)	0.432
HDL cholesterol (mmol/L)	1.50 (0.7-2.2)	1.45 (0.8-2.3)	0.152
LDL cholesterol (mmol/L)	2.55 (1.6-4.4)	2.80 (2.3-4.8)	0.020
Non-HDL cholesterol (mmol/L)	2.90 (1.9-5.3)	3.45 (2.6-6.0)	0.011

Zdenek Racil,[1,2] Filip Razga,[1] Jana Drapalova,[3] Lucie Buresova,[4] Daniela Zackova,[1] Martina Palackova,[1] Lukas Semerad,[1] Ludmila Malaskova,[5] Martin Haluzik,[3] and Jiri Mayer[1,2]

[1]Dept. Internal Medicine, Hematology and Oncology, Masaryk University and University Hospital Brno, Brno; [2]CEITEC - Central European Institute of Technology, Masaryk University Brno, Brno; [3]3rd Dept. Medicine, 1st Faculty of Medicine, Charles University and General University Hospital in Prague, Prague; [4]Institute of Biostatistics and Analyses, Masaryk University Brno, Brno; [5]Department of Clinical Biochemistry, University Hospital Brno, Brno, Czech Republic

Funding: This work was supported by CELL – The CzEch Leukemia Study Group for Life, MH CZ - DRO (FNBr, 65269705), by the CEITEC - CZ.1.05/1.1.00/02.0068 and RVO-VFN64165/2012.

Correspondence: zracil@fnbrno.cz.
doi:10.3324/haematol.2013.086355

Key words: nilotinib, tyrosin kinase inhibitor, glucose metabolism.

The online version of this article has a Supplementary Appendix.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251-9.
2. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care. 1998;21(12):2191-2.
3. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. Diabetes Res Clin Pract. 2000;47(3):177-84.
4. Singh B, Saxena A. Surrogate markers of insulin resistance: A review. World J Diabetes. 2010;1(2):36-47.
5. Phillips LK, Prins JB. Update on incretin hormones. Ann NY Acad Sci. 2011;1243:E55-74.
6. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endoc Relat Cancer. 2009;16(4):1103-23.
7. Frasca F, Pandini G, Malaguarnera R, Mandarino A, Messina RL, Sciacca L, et al. Role of c-Abl in directing metabolic versus mitogenic effects in insulin receptor signaling. J Biol Chem. 2007;282 (36):26077-88.
8. Genua M, Pandini G, Cassarino MF, Messina RL, Frasca F. c-Abl and insulin receptor signalling. Vitam Horm. 2009;80:77-105.
9. Xu A, Tso AW, Cheung BM, Wang Y, Wat NM, Fong CH, et al. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. Circulation. 2007;115(12):1537-43.
10. Shehzad A, Iqbal W, Shehzad O, Lee YS. Adiponectin: regulation of its production and its role in human diseases. Hormones (Athens). 2012;11(1):8-20.
11. Mohlig M, Wegewitz U, Osterhoff M, Isken F, Ristow M, Pfeiffer AF, et al. Insulin decreases human adiponectin plasma levels. Horm Metab Res. 2002;34(11-12):655-8.
12. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia. 2010;53(7):1270-87.
13. Semple RK, Sleigh A, Murgatroyd PR, Adams CA, Bluck L, Jackson S, et al. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. J Clin Invest. 2009;119(2):315-22.
14. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol. 2003;23(1):85-9.
15. Aichberger KJ, Herndlhofer S, Schernthaner GH, Schillinger M, Mitterbauer-Hohendanner G, Sillaber C, et al. Progressive peripheral arterial occlusive disease and other vascular events during nilotinib therapy in CML. Am J Hematol. 2011;86(7):533-9.