Imatinib mesylate improves insulin sensitivity and glucose disposal rates in rats fed a high-fat diet

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ABSTRACT

The aim of the present study was to investigate whether imatinib affects insulin sensitivity and glucose disposal in HF (high-fat)-fed rats. Sprague–Dawley rats were fed either a standard pelleted rat food (low-fat diet) or an HF diet (60% fat) for 8 weeks. During the last 10 days of the HF diet regime, rats received saline alone or imatinib (50 or 100 mg/kg of body weight) daily by gavage. The higher dose of imatinib resulted in a decreased psoas fat pad weight in the HF-treated rats. Under euglycaemic hyperinsulinaemic clamp conditions, HF-fed rats exhibited increased insulin concentrations and decreased glucose disposal. The lower (50 mg/kg of body weight), but not the higher (100 mg/kg of body weight), dose of imatinib normalized insulin sensitivity and glucose disposal without affecting glucose metabolism in low-fat-fed rats. Hepatic glucose production at both fasting and hyperinsulinaemic conditions was only weakly affected by imatinib. We conclude that a moderate dose of imatinib efficiently counteracts HF-induced peripheral insulin resistance, and that further studies on the mechanisms by which imatinib increases insulin action in muscle and fat tissues might generate novel strategies for the treatment of Type 2 diabetes.

INTRODUCTION

Imatinib mesylate, also known as Gleevec or STI571, is a selective tyrosine kinase inhibitor that specifically inhibits c-Abl (cellular Abelson tyrosine kinase), PDGFR (platelet-derived growth factor receptor), the transmembrane receptor tyrosine kinase c-Kit and the Abl-related gene [1,2]. Imatinib is successfully used in the clinic to treat malignancies such as CML (chronic myeloid leukaemia) and GISTs (gastrointestinal stromal tumours) [3,4]. Furthermore, it has been observed that a modest number of patients suffering from both CML and Type 2 diabetes, were successfully treated for not only their leukaemia, but also for diabetes when given imatinib [5,6]. Although the beneficial effect of imatinib in Type 2 diabetes was not reported in a third study [7], also with a modest number of cases, two additional case reports have observed a blood-glucose-lowering effect of imatinib in patients with GISTs [8] and paraneoplastic insulin-resistance syndrome [9]. The molecular mechanisms underlying the beneficial effects of imatinib in these cases are unknown, but we have recently observed that imatinib may counteract diabetes by preserving β-cell viability and mass [10,11]. It appears that imatinib decreases JNK (c-Jun N-terminal kinase) activation in β-cells, which leads to protection against apoptosis [11]. However, as these findings were observed in animal models for Type 1 diabetes, namely the NOD (non-obese diabetic) mouse and the streptozotocin-injected mouse, it is possible that imatinib in Type 2 diabetes also acts by affecting peripheral insulin sensitivity and/or hepatic glucose production. Interestingly, JNK activation, which occurs in peripheral tissues in response to oxidative stress, has been implicated in the pathogenesis of insulin resistance.
and Type 2 diabetes [12]. Thus it might be that imatinib, by preventing JNK activation, not only protects β-cells from apoptosis, but also decreases insulin resistance.

Rats given an HF (high-fat) diet are known to develop obesity, mild hyperglycaemia and decreased insulin sensitivity in muscle, fat and liver [13], and are therefore used as a model of diet-induced obesity and glucose intolerance in humans. In the present study, HF-fed rats were treated with imatinib for 10 days and whole-body insulin action was analysed. Our findings indicate that a moderate dose of imatinib efficiently counteracts the metabolic consequences of the HF diet by lowering whole-body insulin resistance.

**MATERIALS AND METHODS**

**Animals**

Male Sprague–Dawley rats, 4 weeks of age, were purchased from B&K. Animals were weighed at the start of the experiment and weekly thereafter. Blood glucose concentrations were measured each week using an ExacTech blood glucose meter (Medisense). All animal experiments were approved by the Local Animal Ethics Committee for Uppsala University.

**HF diet and imatinib treatment**

Animals had free access to either standard pelleted rat food [LF (low-fat) diet] or an HF diet (60 % fat) (D12492; Research Diets). This HF diet lacks corn starch, is low in sucrose and is high in lard [24.5 % (w/w)]. The HF diet was initiated when rats were 5 weeks of age and continued for a total of 8 weeks. During the last 10 days of the experiment, imatinib was dissolved in water and administered daily by gavage at 50 or 100 mg/kg of body weight. Administration of saline alone daily by gavage was used as a control.

**Preparation of animals for clamp study**

The tenth and final imatinib gavage was given to the rats in the morning of the day of clamp study after an overnight fast. At 3 h later, the rats were then anaesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg of body weight; Mebumal; Apotekbolaget), heparinized and placed on an operating table coupled to thermal pads programmed to maintain a body temperature of 38°C. The anaesthetized rats were tracheotomized and polyethylene catheters were inserted into both femoral veins (connected to peristaltic pumps), one femoral artery (for BP monitoring) and one carotid artery [for BP (blood pressure) monitoring using a PDCR 75/1 pressure transducer (Druck)]. Once the mean arterial BP had stabilized at > 90 mmHg, a 30 min infusion of 0.49 µCi/min D-[3-3H]glucose (Amersham Biosciences) in glucose-free saline was started. During this period, blood glucose was analysed every 10 min, and samples were taken for [3H]glucose determinations every 10 min and for insulin at 0 and 30 min.

**Euglycaemic hyperinsulinaemic clamp procedure**

The clamp was performed essentially as described previously [14,15]. A 30 % (v/v) glucose solution, supplemented with 1 µCi of [3H]glucose/ml, was infused for 60 min at a fixed rate of 27 mg of glucose · min⁻¹ · kg⁻¹ of body weight. Simultaneously, another peristaltic pump delivered human recombinant insulin (Actrapid; Novo Nordisk) at an initial rate of 18 milli-units · min⁻¹ · kg⁻¹ of body weight. The rate of the insulin infusion was adjusted during the beginning of the clamp in order to keep the blood glucose concentration constant at approx. 6 mmol/l. Blood glucose was analysed every 5 min, and blood samples (100 µl) were taken for [3H]glucose determinations every 15 min. Insulin was analysed at 30 and 60 min. After the 60 min clamp, rats were killed and the pancreas and the psoas fat pad were removed and weighed.

**Determination of [3H]glucose activity in plasma**

Blood samples were centrifuged, deproteinized and evaporated as described previously [12]. The samples were then dissolved in 100 µl of water, followed by the addition of 1 ml of Ultima Gold (PerkinElmer) scintillant. 3H counts were quantified in a Wallac 1409 liquid-scintillation counter. Glucose disposal rates were calculated using Steeles equation [16].

**Plasma insulin concentration**

Plasma samples were obtained through centrifugation and analysed for insulin content using an ultrasensitive rat insulin ELISA (Mercodia).

**Statistical analysis**

All results are presented as means ± S.E.M. Significant differences were calculated using one-way or two-way ANOVA, followed by the Student–Newman–Keul post-hoc test.

**RESULTS**

We have observed previously that imatinib counteracts diabetes in mice by promoting β-cell survival [10,11]. To evaluate whether imatinib also counteracts diabetes by interacting with peripheral insulin sensitivity, in the present study, we have analysed the effects of imatinib on glucose production and uptake in rats treated with an HF diet. Briefly, 47 male Sprague–Dawley rats were given standard rat chow (LF diet) or an HF diet for 8 weeks. Out of a total 47 rats, nine were lost or excluded due to gavage to the lung or surgical complications. Among the
nine rats excluded, two were in the LF C group (LF diet without imatinib), three were in the HF 50 group (HF diet + 50 mg of imatinib/kg of body weight), one was in the LF 100 group (LF diet + 100 mg of imatinib/kg of body weight) and three were in the HF 100 group (HF diet + 100 mg of imatinib/kg of body weight).

After 6 weeks of the HF diet, an increase in blood glucose concentration in non-fasted rats was observed (5.29 ± 0.15 and 6.07 ± 0.19 mmol/l glucose in LF- and HF-fed rats respectively; \( P < 0.001 \), as determined using a Student’s \( t \) test). After 6 weeks, the weight of the LF-fed rats was 445 ± 6.1 g, whereas the weight of the HF-fed rats was 491 ± 7.9 g (\( P < 0.001 \), as determined using a Student’s \( t \) test). The HF diet also increased body and psoas fat pad weight after the full 8 weeks of the experiment (Figure 1).

During the last 10 days of this regime, saline or imatinib (at 50 or 100 mg/kg of body weight) was administered daily by gavage. As shown in Figure 1, the HF-diet-induced increase in body weight and psoas fat pad weight were significantly attenuated by the higher dose of imatinib (100 mg/kg of body weight).

Haematocrit and mean arterial BP at the beginning of the procedure and the pancreatic weight at the end of the experiment were also determined (Table 1). Haematocrit was decreased by the higher dose of imatinib in both the LF- and HF-fed rats. The pancreas weight was significantly increased by imatinib in the LF-fed rats (Table 1).

Imatinib treatment did not affect food intake during the last 10 days. A typical food intake during a 3 day period was 144, 144 and 156 g for two LF-fed rats treated with saline, 50 and 100 mg of imatinib/kg of body weight by gavage respectively, and 116, 92 and 119 g for two HF-fed rats treated with saline, 50 and 100 mg of imatinib/kg of body weight respectively.

At 3 h after the final imatinib administration, the overnight-fasted rats were anaesthetized, tracheotomized and catheterized. A 30 min \([3H]\)glucose infusion was performed on the rats, and plasma insulin, blood glucose and whole-body glucose utilization, measured by scintillation counting, were determined. As shown in Figure 2(A), fasting blood glucose concentrations directly after anaesthesia and surgery were unaffected in HF-fed rats compared with LF-fed rats. This is at variance with the results obtained with non-anaesthetized and non-fasted rats (see above), but it is easily envisaged that both overnight fasting and anaesthesia could mask the hyperglycaemic effect of the HF diet. It was also observed that imatinib did not affect the fasting blood glucose concentration of LF-fed rats at time 0; however, in HF-fed rats, imatinib increased fasting blood glucose at both time 0 and after 30 min (Figure 2A). In addition, in LF-fed rats, blood glucose was increased in response to the higher imatinib concentration after 30 min of anaesthesia. Indeed, the mean arterial BP appeared to be higher in imatinib-treated LF-fed rats after anaesthesia (Table 1). Surprisingly, the mean arterial BP was decreased by the lower dose of imatinib in the HF-fed rats.

The imatinib-induced increase in blood glucose in HF-fed rats at 0 min was paralleled by an increase in plasma insulin levels (Figure 2B). In addition, in LF-fed rats, there was a modest increase in plasma insulin levels at the higher imatinib concentration. However, at 30 min, imatinib did not affect insulin levels in either LF- or HF-fed rats. Instead, insulin levels were higher in the HF-fed rats compared with the LF-fed rats.

The glucose disposal rate before the start of the clamp was moderately increased in LF-fed rats treated with the higher dose of imatinib compared with no imatinib treatment (19.8 ± 0.7 compared with 13.6 ± 1.7 mg
Table 1  Haematocrit, mean arterial BP and pancreas weight of rats included in the present study

Haematocrit and arterial BP were determined directly after anaesthetization, surgery and catheterization, i.e. at time 0. The pancreas weight was determined directly after the end of the experiment. Results are means ± S.E.M. *P < 0.05 compared with the corresponding control, as determined by two-way ANOVA and the Student–Newman–Keul test as post-hoc test. C, saline; 50, 50 mg of imatinib/kg of body weight; and 100, 100 mg of imatinib/kg of body weight. Each treatment was administered daily by gavage.

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<td>1.50 ± 0.09</td>
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Figure 2  Blood glucose concentrations (A) and plasma insulin levels (B) at 0 and 30 min after anaesthesia and surgery

Rats were anaesthetized using pentobarbital, and surgery was performed. Blood was taken from a catheter after anaesthesia at the time points indicated, and the glucose concentration was determined using the ExacTech blood glucose meter. Plasma insulin levels were determined using an ultrasensitive rat insulin ELISA. *P < 0.05 compared with the respective control, as determined using one-way ANOVA for repeated measurements and the Student–Newman–Keul post-hoc test. C, saline; 50, 50 mg of imatinib/kg of body weight; and 100, 100 mg of imatinib/kg of body weight. Each treatment was administered daily by gavage.
of glucose \cdot \text{min}^{-1} \cdot \text{kg}^{-1} of body weight respectively; \( P < 0.05 \). This effect was, however, not observed in HF-fed rats (12.2 \pm 0.7 compared with 14.2 \pm 1.2 mg of glucose \cdot \text{min}^{-1} \cdot \text{kg}^{-1} of body weight for rats treated with the higher dose of imatinib compared with no imatinib treatment respectively). The glucose disposal rates were not affected in the rats receiving the lower dose of imatinib (16.8 \pm 0.8 and 13.9 \pm 2.7 mg of glucose \cdot \text{min}^{-1} \cdot \text{kg}^{-1} of body weight for LF- and HF-fed rats receiving 50 mg of imatinib/kg of body weight respectively).

As glucose disposal rates are equal to hepatic glucose output in fasting conditions, it is likely that the imatinib-induced increase in blood glucose resulted, in part, from an increase in hepatic glucose production.

After the 30 min of \([3H]\)glucose infusion under fasting conditions, a euglycaemic hyperinsulinaemic (approx. 6 mmol/l glucose) clamp was initiated. Plasma insulin levels during euglycaemic equilibrium at the end of clamp were significantly higher in HF-fed rats compared with LF-fed rats (Figure 3A). Interestingly, the lower dose of imatinib completely normalized the insulin requirement during the clamp (Figure 3A). An intermediate effect was observed with the higher dose of imatinib. Imatinib did not affect insulin sensitivity in LF-fed rats (Figure 3A).

There were no significant differences in glucose disposal rates between the different groups during the clamp (results not shown). However, as insulin was delivered at different rates and the plasma insulin levels, therefore, were significantly different (Figure 3A) and because glucose disposal rates are known to correlate well with insulin levels [17], we expressed glucose disposal rates per plasma insulin levels normalized to the LF-fed group. In this case, we observed a lowering effect of the HF diet upon glucose disposal rates (Figure 3B), which is in good agreement with the decreased insulin sensitivity observed in Figure 3(A). Interestingly, the lower dose of imatinib increased glucose disposal rates in both LF- and HF-fed rats (Figure 3B). No significant effect was exerted by the higher dose of imatinib.

As the hepatic glucose output rate is equal to the difference between the glucose infusion rate and the glucose disposal rate, we consequently observed that HF-fed rats had increased hepatic glucose output compared with LF-fed rats (Figure 4). The lower dose of imatinib significantly decreased the hepatic glucose output rate in LF-fed rats, but the effect in HF-fed rats did not reach statistical significance.

**DISCUSSION**

In the present study, two doses of imatinib were administered, namely 50 or 100 mg/kg of body weight. In rats, the lower dose, 50 mg/kg of body weight or 0.045 mg/cm² for a rat weighing 500 g, has been reported to result in a peak plasma concentration of approx. 10 \( \mu \text{mol/l} \) [18].

In humans, the maximum plasma imatinib level reaches 4 \( \mu \text{mol/l} \) using the conventional 600 mg dose [19], which corresponds to 0.035 mg/cm² assuming a body weight of 80 kg. It has been observed in pre-clinical rat toxicological studies that most toxicities start to appear at the clinical dose adjusted for body weight [19], indicating that the 100 mg/kg of body weight dose, but probably...
Hepatic glucose output was calculated from the results in Figure 3 and the glucose infusion rate of 13.6 mg/min. *P < 0.05, as determined using one-way ANOVA for repeated measurements and the Student–Newman–Keul post-hoc test. C, saline; 50, 50 mg of imatinib/kg of body weight; and 100, 100 mg of imatinib/kg of body weight. Each treatment was administered daily by gavage.

Figure 4  Hepatic glucose output

Hepatic glucose output was calculated from the results in Figure 3 and the glucose infusion rate of 13.6 mg/min. *P < 0.05, as determined using one-way ANOVA for repeated measurements and the Student–Newman–Keul post-hoc test. C, saline; 50, 50 mg of imatinib/kg of body weight; and 100, 100 mg of imatinib/kg of body weight. Each treatment was administered daily by gavage.

not the 50 mg/kg of body weight dose, may be associated with some toxicity. Indeed, as the higher dose provoked a multitude of effects, including rather dramatic decreases in haematocrit, body weight and psoas fat pad weight, it is likely that most of these changes occurred as the result of toxicity and that damage to other organs, such as the liver and the kidney, might have taken place as well. The complex metabolic alterations observed in the present study in response to the higher dose of imatinib are therefore not easily interpretable.

The lower dose of imatinib did not significantly affect body weight, psoas fat pad weight, haematocrit, BP or fasting blood glucose and insulin levels. Thus we could not observe any signs of toxicity in response to 50 mg of imatinib/kg of body weight. Instead the lower dose of imatinib increased blood glucose and insulin levels of anaesthetized and HF-fed rats. It is not clear why imatinib increases blood glucose and insulin levels during these specific conditions, but it could be speculated that high concentrations of imatinib and an HF diet together augment the anaesthesia/surgery-induced sympathetic stress response. After 30 min of anaesthesia, the insulin level was no longer augmented, which may have resulted from a gradual increase in anaesthesia/surgery-induced inhibition of insulin release [20] or a diminished stress response.

The HF diet given to the rats resulted in increased body weight, psoas fat pad weight, blood glucose and serum insulin concentrations, during both fasting anaesthesia and euglycaemic clamp conditions, and hepatic glucose output, and in decreased glucose disposal rates, all signs typical of diet-induced Type 2 diabetes. The lower dose of imatinib partially or completely counteracted all of these HF-induced effects. These findings suggest that HF-diet-induced whole-body insulin resistance is counteracted by imatinib, and that the drug increases both muscle/fat and liver insulin sensitivity. We cannot exclude a β-cell-protective role of imatinib in the HF-treated rats, but the rather short treatment period (10 days) argues against dramatic changes in β-cell mass and function.

The mechanisms by which imatinib decreases insulin resistance and, to a modest extent, hepatic glucose production are not known. It is possible that the improved insulin sensitivity arises, at least in part, secondary to the slimming effect of imatinib. On the other hand, it appears that Type 2 diabetes patients that were successfully treated for their diabetes with imatinib did not lose weight during treatment [5,6]. An alternative explanation could be that imatinib decreases JNK activation in peripheral tissues, as demonstrated in islets [11]. An HF diet is known to promote mitochondrial ROS (reactive oxygen species) production, which, in turn, leads to increased JNK activity and insulin resistance [21]. Indeed, activation of C-Abi has been demonstrated to result in phosphorylation of MEKK1 [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase 1], which, in turn, promotes M KK4 (MAP kinase 4) and JNK activation [22]. Thus it is conceivable that JNK activation during diet-induced oxidative stress is negatively modulated by the C-Abi inhibitor imatinib. Finally, it has recently been observed that imatinib attenuates PDGFR-induced phosphorylation of LPR (low-density lipoprotein receptor-related protein) [23], which, in turn, might lead to an improved lipoprotein metabolism [24] and protection against atherosclerosis [25]. To what extent this mechanism operates in HF-induced glucose intolerance and the development of Type 2 diabetes remains also to be elucidated.

In conclusion, in the present study, we have observed beneficial effects of a lower imatinib dose (50 mg/kg of body weight) when given to HF-fed rats. On the other hand, the higher dose of imatinib evoked unclear and complicated actions possibly involving toxicity. The side effects of imatinib in the treatment of CML and GISTs preclude imatinib from being used as a therapy in Type 2 diabetes; however, with a better understanding of the mechanisms by which imatinib counteracts diabetes, a novel imatinib-like drug could possibly be generated that exhibits a better side-effect profile.

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REFERENCES


