HYPOTHESIS

Potential utility of small tyrosine kinase inhibitors in the treatment of diabetes

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ABSTRACT

Altered tyrosine kinase signalling has been implicated in several diseases, paving the way for the development of small-molecule TKIs (tyrosine kinase inhibitors). TKIs such as imatinib, sunitinib and dasatinib are clinically used for treating chronic myeloid leukaemia, gastrointestinal stromal tumours and other malignancies. In addition to their use as anti-cancer agents, increasing evidence points towards an anti-diabetic effect of these TKIs. Imatinib and other TKIs counteract diabetes not only in non-obese diabetic mice, but also in streptozotocin diabetic mice, db/db mice, high-fat-treated rats and humans with T2D (Type 2 diabetes). Although the mechanisms of protection need to be investigated further, the effects of imatinib and other TKIs in human T2D and the rapidly growing findings from animal models of T1D (Type 1 diabetes) and T2D are encouraging and give hope to improved treatment of human diabetes. In the present article, we review the anti-diabetic effects of TKIs which appear to involve both protection against β-cell death and improved insulin sensitivity. Considering the relatively mild side effects of TKIs, we hypothesize that TKIs could be used to treat new-onset T1D, prevent T1D in individuals at high risk of developing the disease, treat the late stages of T2D and improve the outcome of islet transplantation.

INTRODUCTION

Patients diagnosed with T1D (Type 1 diabetes) have usually lost a substantial proportion of their β-cells and therefore their ability to maintain sufficient insulin production. Although the disease onset can be precipitous, in most cases the disease process appears to start years before the actual clinical outcome. During this long pre-clinical period, patients usually do not display any prominent symptoms, although the presence of autoantibodies to β-cells and their antigens can be detected at an early stage. Dysfunction and damage of β-cells in the early phase is thought to result from a direct contact with islet-infiltrating cells (macrophages and CD4+/CD8+ T-cells) and/or exposure to cytotoxic mediators [for example pro-inflammatory cytokines, ROS (reactive oxygen species) and Fas ligand] produced by these cells [1].

In the later stages of T2D (Type 2 diabetes), β-cells are also dysfunctional and damaged, possibly in response to peripheral insulin resistance, hyperglycaemia, hyperlipidaemia and cytokines, leading to a relative lack of insulin [2]. The molecular events leading to cytokine-induced β-cell dysfunction and death have been investigated and it appears that the activation of the transcription factors NF-κB (nuclear factor κB) and STATs (signal transducers and activators of transcription) and the MAPKs (mitogen-activated protein kinases), such as JNK (c-Jun N-terminal kinase) and p38 MAPK, in response to both cytokines and oxidative stress, plays a central role in this chain of events [3].

Key words: β-cell, diabetes, imatinib, c-Abl, platelet-derived growth factor receptor (PDGFR), tyrosine kinase inhibitor.
Abbreviations: Arg, Abl-related-gene; ATM, ataxia telangiectasia mutated; CML, chronic myeloid leukaemia; DDR, discoidin domain receptor; ER, endoplasmic reticulum; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NOD, non-obese diabetic; NQO2, NADPH dehydrogenase quinone 2; PDGFR, platelet-derived growth factor receptor; SCF, stem cell factor; STZ, streptozotocin; T1D, Type 1 diabetes; T2D, Type 2 diabetes; TKI, tyrosine kinase inhibitor.
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Several strategies have been tested to alleviate diabetes in humans. Current treatments for T2D include exercise, diet control, gastric bypass surgery and a variety of drugs affecting insulin sensitivity and release (biguanides, glitazones, sulfonylureas and activators of incretins/incretin receptors). Despite the many options in the treatment of T2D, many patients still develop the typical complications associated with diabetes. A limited number of T1D cases have successfully been treated by islet or pancreas transplantation. The remaining T1D patients are doomed to life-long insulin therapy. Pharmacological approaches to prevent or reverse the development of T1D have mostly been aimed at intervening in the autoimmune process, thereby preserving the β-cells (cyclosporine, nicotinamide, oral/nasal insulin, GAD 65 peptide and anti-CD3 monoclonal antibody treatment). Although interesting and valuable findings have emerged from these prevention trials, the treatments had either little effect on the development of T1D or unwanted side effects that predominated over the anti-diabetic effects [4].

Abnormal tyrosine kinase signalling has been implicated in several diseases, paving the way for the development of small-molecule TKIs (tyrosine kinase inhibitors). In 2002, the TKI imatinib (Gleevec®; STI-571) was approved for clinical treatment of CML (chronic myeloid leukaemia). Today, imatinib and its successor nilotinib (Tasigna®) are used as treatments of CML, GISTs (gastrointestinal stromal tumours) and other malignancies. CML is characterized by a chromosomal translocation resulting in uncontrolled tyrosine kinase activity of the fusion protein Bcr-Abl [5]. Imatinib is a 2-phenylaminopyrimidine-based ATP-competitive inhibitor of the Abl protein kinase and was created using the structure of the ATP-binding site of the kinase. Imatinib binds to and stabilizes the inactive form of Bcr-Abl, diminishing the effects of the Bcr-Abl oncoprotein through the inhibition of autophosphorylation and substrate phosphorylation [5]. Nilotinib has a similar target specificity as imatinib, but is 20–50-fold more potent than imatinib against Bcr-Abl [5]. The search for additional molecular targets revealed that imatinib and nilotinib also inhibit Arg (Abl-related-gene), the SCF (stem cell factor) receptor c-Kit, PDGFR (platelet-derived growth factor receptor), DDR1/2 (discoidin domain receptor 1/2) and the oxidoreductase NQO2 (NADPH dehydrogenase quinone 2) at sub-micromolar concentrations (Table 1) [6].

TKIs can be taken orally, making it possible for the patient to self-administer the drug daily. In CML therapy, imatinib is given in standard doses of 400 or 600 mg/day. Most of the patients respond well to these doses; however, primary resistance to imatinib occurs in 2–4 % of all chronic CML patients and is mostly linked to mutations in the Bcr-Abl kinase. Imatinib treatment is in most cases well-tolerated. The most common adverse effects, affecting 40–60 % of the CML patients receiving imatinib treatment, are dose-dependent but transient, and include oedema, nausea, diarrhoea, rash and other skin problems. Some patients experience a mild depression of the bone marrow as evidenced by neutropenia (17 %), thrombocytopenia (9 %) and anaemia (4 %). A few sporadic cases of cardiovascular, liver or kidney toxicity have been reported [7].

The anti-diabetic effects of TKIs have recently been reviewed by Little at al. [8]. In the present article, we hypothesize that TKIs have anti-diabetic effects in both T1D and T2D, and that this could be mediated by both improved β-cell survival and decreased insulin resistance. We summarize the increasing evidence supporting our hypothesis and discuss the mechanisms that may be involved in mediating the protection against diabetes. Although there have been a number of case reports supporting the beneficial effects of different TKIs in diabetes [9–17], we focus on imatinib, which is the best-studied TKI, and its potential for the treatment of both T1D and T2D.

EFFECTS OF IMATINIB AND OTHER TKIs IN ANIMAL MODELS WITH ENHANCED β-CELL DESTRUCTION

Imatinib prevents and ameliorates diabetes in animal models characterized by β-cell dysfunction/destruction. We have observed that imatinib prevents β-cell death \textit{in vitro} and diabetes in NOD (non-obese diabetic) mice and STZ (streptozotocin) diabetic mice, which are two models for human β-cell destruction and T1D [18,19]. The NOD mouse is a genetic model of T1D in which β-cells are destroyed by islet-infiltrating immune cells [20]. In the STZ diabetic mouse, STZ specifically kills β-cells, which results in overt hyperglycaemia [21]. In a more recent publication, imatinib and a second-generation TKI, sunitinib, both not only prevented, but also reversed new-onset diabetes in NOD mice [22]. Interestingly, a 10-week treatment period with imatinib resulted in protection against the recurrence of disease for an extended period after discontinuation of imatinib therapy [22]. Imatinib treatment has also been evaluated in the \textit{db/db} mouse, which develop β-cell destruction by a non-autoimmune mechanism. In a study from 2009 [23], imatinib induced the remission of diabetes in these mice, possibly via increasing β-cell mass by preventing β-cell apoptosis and increasing β-cell proliferation. In addition to the results obtained in animal models, a number of case reports have shown that imatinib mitigates other autoimmune diseases such as rheumatoid arthritis and Crohn’s disease in humans [24,25].

Although there exists proof-of-principle for an anti-diabetic effect of imatinib and similar TKIs in animal models of T1D and β-cell destruction, the mechanisms by which they protect against diabetes are not clear,
Table 1 TKIs and their reported protective effect in diabetes from animal models and case reports

<table>
<thead>
<tr>
<th>TKI</th>
<th>Known targets</th>
<th>Animal models of β-cell destruction</th>
<th>Animal models of insulin-resistance</th>
<th>Case reports</th>
</tr>
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<tbody>
<tr>
<td>Imatinib (Gleevec&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>c-Abl, Arg, c-Kit, DDR1/2, PDGFR and NQO2</td>
<td>Protects against STZ-induced diabetes &lt;sup&gt;[19]&lt;/sup&gt;; prevents and reverses diabetes in NOD mice &lt;sup&gt;[18,22]&lt;/sup&gt;; induces remission of diabetes in db/db mice &lt;sup&gt;[23]&lt;/sup&gt;</td>
<td>Reduces high-fat diet-induced insulin resistance &lt;sup&gt;[28]&lt;/sup&gt;</td>
<td>Remission of diabetes in CML patients &lt;sup&gt;[9,10]&lt;/sup&gt;; improved insulin sensitivity in non-diabetic CML patients &lt;sup&gt;[14]&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sunitinib (Sutent&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>VEGFR, c-Kit, PDGFR and Flt3</td>
<td>Reverses diabetes in NOD mice &lt;sup&gt;[22]&lt;/sup&gt;</td>
<td></td>
<td>Remission of diabetes in renal cell carcinoma patients &lt;sup&gt;[12]&lt;/sup&gt;</td>
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<tr>
<td>Dasatinib (Sprycel&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>c-Abl, Arg, Src, PDGFR, c-Kit, Yes, Fyn, Lyn etc.</td>
<td>Improved fasting glucose in a CML patient with T2D &lt;sup&gt;[13]&lt;/sup&gt;</td>
<td></td>
<td>Lowered blood glucose levels in a lung cancer patient &lt;sup&gt;[15]&lt;/sup&gt;</td>
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<tr>
<td>Erlotinib (Tarceva&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>EGFR</td>
<td>Lowered blood glucose levels in a lung cancer patient &lt;sup&gt;[15]&lt;/sup&gt;</td>
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<td>Fasudil</td>
<td>Rhe-kinase</td>
<td>Prevents diabetes in insulin-resistant rats &lt;sup&gt;[17]&lt;/sup&gt;</td>
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However, some possible mechanisms of action have been proposed and they are discussed below.

**EFFECTS OF IMATINIB AND OTHER TKIs IN ANIMAL MODELS CHARACTERIZED BY INSULIN RESISTANCE AND T2D PATIENTS**

Imatinib and other TKIs have also been reported to have beneficial effects in the setting of T2D. In 2004, it was reported that patients diagnosed with both CML and T2D were cured from not only leukaemia, but also diabetes, when treated with imatinib <sup>[9,10]</sup>. Although two subsequent studies found no effect of imatinib on T2D <sup>[26,27]</sup>, other additional studies report anti-hyperglycaemic/anti-diabetic effects of imatinib and similar TKIs in humans <sup>[11–17]</sup>. An anti-diabetic action of imatinib in T2D is supported further by our recent observation that imatinib counteracts high-fat diet-induced insulin resistance and hyperglycaemia in rats <sup>[28]</sup>. Thus, in both an animal model and in T2D patients, imatinib appears to improve glycaemic control, possibly via an insulin-resistance-lowering effect.

**POSSIBLE MECHANISMS FOR THE ANTI-DIABETIC EFFECTS OF IMATINIB**

First, imatinib could protect against diabetes via inhibition of c-Abl. The complex structure of c-Abl infers that this protein can sense and integrate information from multiple signalling pathways and then interact with downstream effector proteins either by phosphorylating tyrosine residues or by binding to strategic target sites. Under physiological conditions, c-Abl has been shown to participate in the control of cytoskeletal function, such as migration and cell structure, and cell cycle progression. However, when cells are exposed to different forms of stress, c-Abl becomes highly activated, which leads to cell cycle arrest and apoptosis. Genotoxic-induced apoptosis appears to require nuclear c-Abl, whereas apoptosis in response to oxidative stress and ER (endoplasmic reticulum) stress is mediated by cytosolic c-Abl <sup>[29,30]</sup>. It appears that the ATM (ataxia telangiectasia mutated) protein, DNA-dependent protein kinase or PKC<sub>ε</sub> (protein kinase C<sub>ε</sub>) activate c-Abl by phosphorylation, and this leads to activation of the stress-activated protein kinases (JNK and p38 MAPK) and the tumour suppressors p53 and p73 <sup>[31,32]</sup>. In addition, anti-apoptotic pathways, such as Bcl-X<sub>L</sub>, NF-κB and PI3K (phosphoinositide 3-kinase) are inactivated <sup>[33,34]</sup>. In certain cells, c-Abl re-localizes upon activation to the mitochondria, which results in a loss of mitochondrial membrane potential, release of cytochrome c and cell death <sup>[35]</sup>. A substantial proportion of c-Abl appears to reside at the ER under normal conditions. It is not until cells are subjected to ER stress that c-Abl is targeted to the mitochondria and promotes either necrosis or apoptosis <sup>[36]</sup>. Interestingly, NO has been reported to induce ER stress in insulin-producing cells <sup>[37]</sup>. In addition, it has been observed that c-Abl binds to the ER protein Aph2, an event that appears to promote apoptosis <sup>[38]</sup>. Thus it is likely that c-Abl is activated in stressed β-cells and this event promotes β-cells apoptosis. Indeed, treatment of β-cells with imatinib protected against the β-cell toxins STZ, DETA/ONOate (diethylenetriamine diazenium-diolate) and H<sub>2</sub>O<sub>2</sub> in vitro <sup>[18,19]</sup>. Moreover, the effect of imatinib was mimicked by c-Abl siRNA (small interfering RNA), strengthening the idea that imatinib protects against β-cell death by interfering with c-Abl signalling <sup>[18,19]</sup>. The imatinib-induced protection was
paralleled by a lowered activation of the pro-apoptotic MAPK JNK [19] and an increased activation of the anti-apoptotic transcription factor NF-κB [18,19]. It has also been observed that imatinib decreases β-cell ER stress in the diabetic db/db mouse [23], and, as c-Abl has been suggested to participate in ER-stress-induced cell death [36], it is possible that imatinib acts via amelioration of ER stress. Furthermore, imatinib has been demonstrated to affect insulin signalling and downstream metabolic and proliferative events [39]. Imatinib treatment was found to blunt insulin-induced Akt/protein kinase B activation and augment the activation of ERK (extracellular-signalregulated kinase) in a hepatoma cell line, an effect that was specific to c-Abl inhibition [39]. Therefore c-Abl inhibition could also protect against diabetes in part by increasing proliferatory signals.

Secondly, imatinib and other TKIs may act by inhibition of PDGFR. Louvet et al. [22] reported that imatinib and sunitinib both prevented and reversed established diabetes in NOD mice. As the effect was partially mirrored by a PDGFR trap (consisting of the extracellular domain of PDGFRβ fused to the Fc domain of human IgG), but not by agents targeting c-Kit, and that sunitinib, which is not known to inhibit c-Abl, also reversed new-onset diabetes, the authors proposed that the effect was mainly mediated by the inhibition of PDGFR. It is known that PDGF signalling is enhanced in arteriosclerosis and diabetes, possibly via high glucose [40], low PPARγ (peroxisome-proliferator-activated receptor γ), ApoE (apolipoprotein E) and adiponectin [41–43], and that the enhanced PDGF signalling results in not only arteriosclerosis, but also possibly worsened insulin resistance [44]. It has been observed that imatinib protects against diabetes-associated arteriosclerosis [45] and autoimmune nephritis in a mouse model of lupus [46], probably via PDGFR inhibition, and it is conceivable that imatinib-mediated protection against insulin resistance and diabetes, at least in part, involves inhibition of this pathway.

Thirdly, the TKIs imatinib, nilotinib sunitinib and dasatinib are known to inhibit c-Kit (Table 1). The ligand for c-Kit, SCF, is an important growth factor for mast cells, promoting their generation from CD34+ progenitor cells [47]. SCF also controls mast cell survival and release of pro-inflammatory cytokines and chemokines. Indeed, mast cells have been suggested to play a central role in the development of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, Guillain–Barre’s syndrome and T1D [48].

SCF also induces eosinophil adhesion and activation, and is up-regulated in inflammatory conditions, both in vitro and in vivo in humans and mice [47]. This indicates that inhibition of the SCF/c-Kit pathway may affect the inflammatory component of both T1D and T2D.

Fourthly, it has recently been observed that imatinib and nilotinib inhibit collagen-induced DDR1/2 [49]. DDR1 is an extracellular matrix receptor that has been reported to convey signals pertinent to the immune system and inflammatory reactions [50]. Thus inhibition of this signalling pathway might dampen diabetes-associated inflammation.

Taken together, the above-mentioned studies indicate that imatinib counteracts diabetes via different molecular mechanisms (Figure 1). For example, imatinib may enhance β-cell survival by decreasing JNK activation and ER stress, and enhancing NF-κB. Peripheral insulin resistance may be ameliorated by imatinib via the inhibition of excessive PDGFR signalling. Decreased insulin resistance in T1D could result in β-cell rest and protection from immunity. Inflammation and innate immunity may be dampened by imatinib through inhibition of c-Kit and DDR1/2. The relative importance and contribution of these different mechanisms in the development of human diabetes, however, are not known.

**NEXT STOP: CLINICAL TRIALS**

The effects of imatinib and other TKIs in human T2D and the rapidly growing results from T1D/T2D animal models give hope to improved treatment of human diabetes. It should be noted that promising results obtained from NOD mice have failed to reach clinical success in the past. However, imatinib and other TKIs are unique in their ability to counteract diabetes not only in NOD mice, but also in STZ diabetic mice, db/db mice, high-fat diet-treated rats and humans with T2D. Therefore only appropriate clinical trials can answer the question of whether imatinib or other TKIs could be used in the treatment of T1D or T2D. We therefore propose the following list of specific hypotheses with appropriate clinical trials for validation/rejection. (i) TKIs prevent β-cell destruction in new-onset T1D patients. This hypothesis can be tested by giving a TKI to T1D patients with a history of diabetes of <3 months. Most patients with a short history of T1D still have some surviving β-cells and, if these are saved from continued destruction, the course of the disease may be reverted. The TKI should be given for 3 months only since, as indicated by results in the NOD mice, it is possible that a time-limited treatment regime with a TKI will not only revert new-onset T1D, but also induce a long-term remission. The effect of the treatment on insulin requirement, β-cell function and insulin resistance should be analysed at 3, 6 and 9 months after the start of treatment. (ii) TKIs attenuate inflammatory and autoimmune events in pre-diabetic individuals so that precipitation of T1D is delayed/prevented. Individuals with a high risk of developing T1D can be identified by analysis of family history, genetic markers and antibody titres. Such individuals should be given a 3-month TKI treatment and the effects of the treatment evaluated by assessments of β-cell function, antibody titres, inflammatory markers and time to precipitation of disease. (iii) TKIs...
Imatinib is known to inhibit the tyrosine kinases c-Abl, PDGFR, c-Kit and DDR1/2. Most likely, imatinib-induced protection against diabetes is mediated not by one single pathway, but via different molecular mechanisms. β-Cell survival is promoted by inhibition of c-Abl, which leads to decreased activation of the pro-apoptotic MAPK JNK and increased activation of the anti-apoptotic transcription factor NF-κB. c-Abl inhibition might also lead to a dampened ER-stress response, via JNK or other pathways. Inhibition of PDGFR could contribute to decreasing peripheral insulin resistance and inflammatory processes, thereby promoting β-cell survival. Moreover, inhibition of c-Kit and DDR1/2 might also add to the anti-diabetic effects of imatinib, possibly by interfering with inflammatory responses.

improve β-cell function and insulin sensitivity in late decompensation stages of T2D so that insulin therapy is not required. The adverse effects of a 3-month TKI treatment are probably less severe than the complications associated with insulin-requiring T2D. Therefore insulin-requiring T2D patients should be given a TKI for 3 months. After 3, 6 and 9 months, metabolic parameters should be analysed and standard tests for insulin resistance and β-cell function performed. (iv) TKIs protect transplanted islets from different forms of post-transplantation stress, so that the effect of the transplantation is improved and prolonged. It is known that a large proportion of β-cells are destroyed when transplanted into diabetic recipients. Factors such as complement activation, hypoxia, poor engrafting, inflammation, recurrence of autoimmune destruction and toxicity of antirejection drugs all promote the loss of β-cells. Assuming that TKIs enhance β-cell survival, a better outcome of the transplantation may be possible. To test this hypothesis, islet-transplanted T1D patients should be given a TKI for 3 months following the transplantation, and the metabolic parameters of the patients followed for another 3, 6 and 9 months.

In addition to the clinical trials proposed above, it is highly warranted to continue pre-clinical investigations aiming at the discovery of novel targets and actions of mechanisms of imatinib and other TKIs. By intensifying our efforts to understand more closely the mechanisms by which TKIs elicit their anti-diabetic effects, it is possible that our understanding of the disease process would improve, which could promote the development of new therapeutic agents.

REFERENCES