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Imeglimin Preserves β-cell Function and Mass in Male Zucker Diabetic Fatty Rats

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Abstract

Aims

Progressive insulin resistance and loss of β-cell function and mass are primary defaults in type 2 diabetes mellitus. Imeglimin has been shown to decrease β-cell apoptosis against various acute stresses (high glucose; cytokines). The purpose of this study was to investigate Imeglimin effect on the delay in onset and subsequent control of diabetes progression in a type 2 diabetes model, the Zucker Diabetic Fatty (ZDF) rat.

Methods

7-week-old ZDF rats were treated orally with Imeglimin 150 mg/kg bid or vehicle for 5 weeks. Glucose tolerance, pancreatic insulin content, β-cell mass, apoptosis and proliferation were measured at the end of treatment.

Results

Imeglimin treatment induced a significant improvement in glucose tolerance (−33%, p<0.01) and increased insulin levels both in the basal state (+72%, p<0.05) and in response to glucose (+77%, NS) compared with controls. The insulinogenic index ΔI/ΔG (+165%, p<0.01) was increased, demonstrating Imeglimin benefit on β-cell function. In parallel, Imeglimin increased pancreatic insulin content (+109%, p<0.0581).

An abnormal islet architecture was observed in ZDF control pancreases, while Imeglimin treatment preserved islet architecture and significantly increased β-cell mass (+41%, p<0.01). In addition, Imeglimin significantly decreased the proportion of apoptotic β cells within islets (−52% vs ctrl, p<0.05) and significantly increased the proportion of proliferative β cells (+111% vs ctrl, p<0.001).

Conclusion

Imeglimin improved β-cell function and slowed down disease progression by preserving β-cell mass and islet architecture with a decrease in β-cell apoptosis and an increase in β-cell proliferation in ZDF rat.

Background

- Insulin resistance and β-cell dysfunction are the primary pathophysiological mechanisms underlying type 2 diabetes mellitus[1]
- Imeglimin is the first in a new tetrahydrotriazine-containing class of oral glucose-lowering agents, the Glimins[2]
- The unique mechanism of action of Imeglimin involves the regulation of mitochondrial bioenergetics; this leads to a potentiation of glucose-stimulated insulin secretion and to an improvement of insulin sensitivity, so targeting the two critical defects at the root of type 2 diabetes[3,4]
- Imeglimin was also shown to protect both β cells and endothelial cells from death induced by oxidative stress and to improve β-cell function[5–7]

Objectives

- The aim of this study was to investigate the *in vivo* effect of a 5-week Imeglimin treatment on the delay in onset of type 2 diabetes mellitus using the Zucker Diabetic Fatty (ZDF) rat model, with a particular focus on the impact of Imeglimin on pancreatic β cells

Research Methods

Animal model and study design

- Male ZDF rats were purchased from Charles River Laboratories (Domaine des Oncins – 69592 L'Arbresle France). All animals were housed in a temperature-controlled environment under constant humidity on a 12-hour light–dark cycle (light on at 07:00) and access to food and water *ad libitum*. All experiments on animals were carried out in accordance with the European animal care guidelines (ETS 123)
- The 7-week-old ZDF rats were randomized into two groups of 20 rats, each to receive 150 mg/kg of Imeglimin (Group 1) or vehicle (methylcellulose 0.5%; controls) orally twice daily for 5 weeks
- Pancreatic tissue samples were collected on Day 37; tissue collection was carried out 1 hour after morning administration of Imeglimin/vehicle in 3-hour-fasted rats

Assessment of glucose tolerance

- Oral glucose tolerance test (OGTT) was carried out on Day 35, 1 hour after morning administration in 3-hour-fasted rats. A first blood sample was collected at T0 before the oral glucose load (2 g/kg, 5 mL/kg). Subsequent blood samples were collected at T+10, T+20, T+30, T+60 and T+120 min after the glucose load. All plasma samples were frozen and stored at −20°C until glucose and insulin measurement
- Glucose and insulin measurements were performed using commercially available kits (glucose: A11A01667, ABX diagnostics, CA, USA; insulin: 80-INSTRU-E01, Alpco, NH, USA)

Assessment of pancreatic insulin content

- Pancreatic insulin content was measured in homogenized pancreatic tissue samples using a commercially available kit (80-INSTRU-E01, Alpco, NH, USA)

Measurement of β-cell mass, proliferation and apoptosis

- Histological analysis was performed in fixed, dehydrated and paraffin-embedded 7-µm-thick sections of pancreatic tissue. Assessment of β-cell mass, proliferation and apoptosis was carried out in at least eight sections randomly chosen every 100–250 µm throughout the block
- To assess the β-cell mass, the slices were stained with guinea pig anti-insulin antibody and, for each slice, the relative cross-sectional area of the β cell was determined by quantifying the cross-sectional area occupied by β cells and dividing it by the cross-sectional area of total tissue per slice
- To determine β-cell proliferation, sections were immuno-stained twice with a rabbit antiKi-67 antibody and a polyclonal guinea pig anti-insulin antibody, following which slices were imaged using an image-analyzing system to determine the number of cells stained with both insulin and Ki-67
- To evaluate β-cell apoptosis, fixed pancreatic sections were incubated with a polyclonal rabbit anti-caspase 3 antibody and with a polyclonal guinea pig anti-insulin antibody; the number of cells stained with both insulin and caspase-3 were determined using an image analyzing system

Statistical analysis

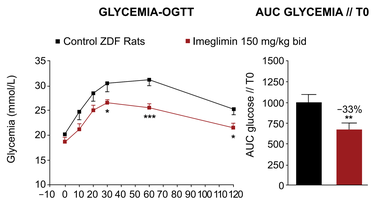
- Statistical analysis was performed using analysis of variance followed by a Dunnett t-test. Differences were considered significant at p<0.05. Results are presented as the mean ± standard error of the mean, together with the number of individual observations (n)

Results

Imeglimin treatment improves glycemia and β-cell function in ZDF rats

- A 5-week treatment with Imeglimin (150 mg/kg) substantially improved glucose tolerance in ZDF rats compared with controls, as evidenced by a significant decrease in the area under the curve (AUC) glucose measured using OGTT (−33%, p<0.01) (**Figure 1a and b**)

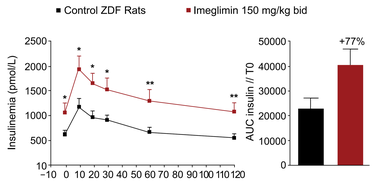
Figure 1a and b: Imeglimin improved glucose tolerance after 5 weeks of treatment



Student t-test / Mann-Whitney: *p<0.05, **p<0.01, ***p<0.001
OGTT 3-hour-fasted
AUC, area under the curve; bid, twice daily; OGTT, oral glucose tolerance test;

- Imeglimin treatment significantly increased basal insulinemia by 72% (p<0.05) (**Figure 2a**)
- Imeglimin treatment increased AUC insulinemia (AUC insulin/T0) compared with controls (+77%, NS) (**Figure 2a and b**)

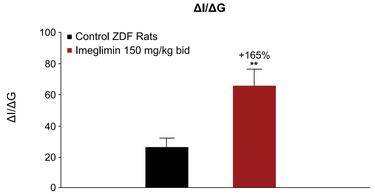
Figure 2a and b: Imeglimin increased insulin secretion in response to glucose after 5 weeks of treatment



Student t-test / Mann-Whitney: *p<0.05, **p<0.01 vs Control
OGTT 3-hour-fasted
AUC, area under the curve; bid, twice daily; OGTT, oral glucose tolerance test

- Imeglimin treatment strongly and significantly improved the insulinogenic index (ΔI/ΔG) after 5 weeks of treatment versus controls (+165%, p<0.01) (**Figure 3**)

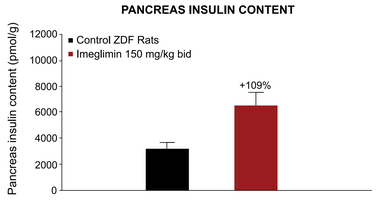
Figure 3: Imeglimin increased the insulinogenic index after 5 weeks of treatment



Student t-test / Mann-Whitney: **p<0.01 vs Control
OGTT 3-hour-fasted
bid, twice daily

- Imeglimin treatment increased pancreatic insulin content by 109% (p<0.0581) compared with controls (**Figure 4**)

Figure 4: Imeglimin increased pancreatic insulin content after 5 weeks of treatment

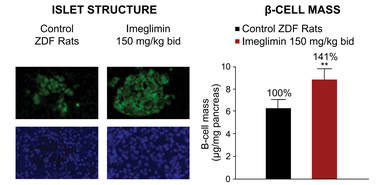


Dunnett t-test: p<0.0581
bid, twice daily

Imeglimin treatment slows down the progression of pathophysiological mechanisms underlying type 2 diabetes mellitus in ZDF rats

- Histological assessment of pancreatic islets showed that Imeglimin preserved islet structure compared with control ZDF rat pancreases, which demonstrated an irregular islet architecture (**Figure 5a**)
- Imeglimin treatment led to a significantly higher β-cell mass (**Figure 5b**)

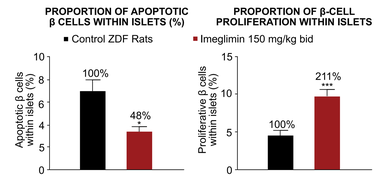
Figure 5a and b: Imeglimin preserved islet architecture and increased β-cell mass after 5 weeks of treatment



Dunnett t-test: **p<0.01 vs Control
Pancreas sections immunostained with guinea pig anti-insulin antibody (green) and nuclei labeled with DAPI (blue)

- Imeglimin had a significant beneficial effect on pancreatic β cells, reducing the proportion of apoptotic cells by more than 50% (−52%, p<0.05) and significantly increasing the proportion of proliferating cells compared with controls (+111%, p<0.001) (**Figure 6a and b**)

Figure 6a and b: Imeglimin decreased islet β-cell apoptosis and increased β-cell proliferation after 5 weeks of treatment



Kruskal-Wallis followed by Dunnett t-test: *p<0.05
bid, twice daily

Mann Whitney's test: ***p<0.001

Conclusions

- Imeglimin, a novel drug candidate, has demonstrated beneficial effects on glucose tolerance and β-cell function *in vivo* in young male ZDF rats, improving both pancreatic insulin content and insulin secretion in response to glucose compared with ZDF controls rats
- Imeglimin slowed down disease progression by preserving β-cell mass and islet architecture with a decrease in β-cell apoptosis and an increase in β-cell proliferation in ZDF rats
- These data demonstrate that Imeglimin may be beneficial in delaying the development of type 2 diabetes mellitus

Acknowledgments

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Conflicts of Interests

Sophie Hallakou-Bozec and Sébastien Bolze are employees of Poxel SA

References

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