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Imeglimin Improves Vascular Dysfunction in Type 2 Diabetes Animal Models

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Background

- Imeglimin is the first in a new tetrahydrotriazine-containing class of oral antidiabetic agents, the glimins[1]
- 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[2,3]
- Imeglimin was also shown to protect both beta cells and endothelial cells from death induced by oxidative stress and to improve beta cell function[4–6]
- Endothelial dysfunction is the first step in the development of vascular disease, leading to an impairment in vascular tone and reactivity, mainly associated with an imbalance between endothelium-derived vasodilatory and contractile factors[7]

Objectives

The aim of this study was to investigate the effects of Imeglimin on endothelial dysfunction in diabetic mice

Research Methods

***Effect of Imeglimin on cutaneous microcirculation* in vivo**

- Fasted male Swiss mice (20–30g) were given a single intraperitoneal injection of streptozotocin (STZ; 200 mg/kg), resulting in hyperglycemia 2 days later. Mice with a blood glucose over 300 mg/dL were included in the diabetic group (Figure 1)
- During a 1-week period, STZ mice were treated with one of three doses of Imeglimin twice daily (75, 150 or 300 mg/kg) or vehicle (control STZ mice) (Figure 1)

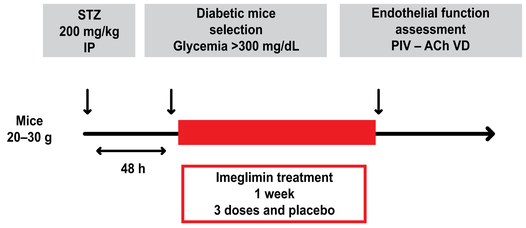

Figure 1. Study design for *in vivo* assessment of endothelial function
ACh, acetylcholine; IP, intraperitoneal; PIV, pressure-induced vasodilation; STZ, streptozotocin; VD, vasodilation

- Endothelial dysfunction was assessed by both pressure-induced vasodilation (PIV) and by acetylcholine (ACh)-induced vasodilation
- PIV was assessed by measuring skin blood flow in response to local pressure using Laser Doppler Flowmetry (LDF). Pressure was progressively increased by 2.2 Pa/s through the Doppler probe, and the LDF signal was averaged every 30 s to reduce the instantaneous variability of the signals as a result of vasomotion (Figure 2a and 2b)

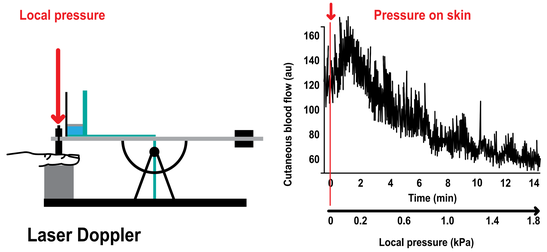

Figure 2a and 2b. Schematic showing weighbridge to hold Doppler probe and effect on cutaneous blood flow as local pressure increases
au, arbitrary unit

- ACh-induced vasodilation was assessed by continuously recording skin blood flow for 15 min using a Laser Doppler multifiber probe (Perimed, Sweden) during transcutaneous iontophoretic delivery of ACh using anodal current application (100 µA for 10 s)

***Effect of Imeglimin on contraction and relaxation of aortic rings* in vitro** (Figure 3)

- The thoracic aorta was excised from both healthy control mice and db/db mice that modeled obesity, diabetes, and dyslipidemia. The vessels were bathed in isolated organ chambers
- The tension of the aortic rings was optimized during a 60 min equilibration period and they were then repeatedly activated with 80 mmol/L KCl to standardize the contractile response
- Imeglimin's effect on vascular reactivity was assessed using phenylephrine (PE)-induced contraction
- Imeglimin (250 µM or 1 mM) or vehicle were added to the organ bath for 30 minutes and cumulative concentration-response curves were constructed with PE incremental concentrations from 1 nM to 10 µM

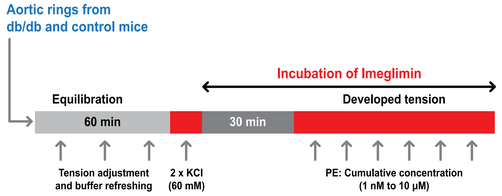

Figure 3. Study design for *in vitro* experiment on aortic rings from normal and diabetic mice
KCl, potassium chloride; PE, phenylephrine

Animal set: 12 db/db mice and 12 control mice (n=8–12 rings/group):

1/ Control mice + vehicle
2/ Control mice + Imeglimin (250 µM)
3/ Control mice + Imeglimin (1 mM)
4/ Diabetic mice + vehicle
5/ Diabetic mice + Imeglimin (250 µM)
6/ Diabetic mice + Imeglimin (1 mM)

Results

Imeglimin prevents abolition of ACh- or PIV-induced vasodilation in STZ mice

- In the non-treated STZ group, the endothelium-dependent vasodilation was reduced compared with the non-diabetic mice, both for PIV (42±8% vs –8±5%, *P*<0.01) and ACh-induced vasodilation (61±15% vs 25±4%, *P*<0.05) (Figure 4a and 4b)
- Imeglimin, at the 2 higher doses, improved PIV (60±9% Imeglimin 150 mg/kg, *P*<0.01 and 35±7% Imeglimin 300 mg/kg, *P*<0.01) and ACh-induced vasodilation (50±7% Imeglimin 150 mg/kg, *P*<0.01 and 40±6% Imeglimin 300 mg/kg) in STZ mice compared with non-treated STZ control animals, restoring close-to-normal response compared with the non-diabetic group (Figure 4a and 4b)
- In contrast, the lowest dose of Imeglimin (75 mg/kg) had no effect on PIV and ACh-induced vasodilation compared with the non-treated STZ mice (Figure 4a and 4b)

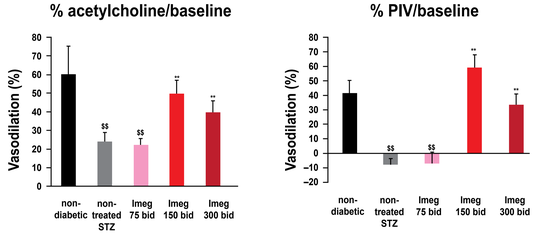

Figure 4a and 4b. Imeglimin increases ACh-dependent vasodilation and PIV response
ACh, acetylcholine; bid, twice daily; Imeg, Imeglimin; PIV, pressure-induced vasodilation; STZ, streptozotocin; $^{\$\$}P$<0.01 vs non-diabetic mice; $^{**}P$<0.01 vs non-treated STZ mice

Imeglimin reduced aortic PE-induced contractility in a dose-dependent manner

- Cumulative concentrations of PE caused a concentration-dependent contraction in both control and diabetic mice. PE-induced contractility was significantly increased in diabetic mice compared with control mice with 1.4-fold increase maximal effect (Figure 5)
- For both diabetic and control mice, incubation with Imeglimin reduced the sensitivity of aortic rings to PE in a dose-dependent manner, with Imeglimin at 1 mM reducing the enhanced reactivity to PE to the level obtained in control animals (Figure 5)
- Imeglimin had a dose-dependent effect on PE-induced contractility; however, the effect of both ACh or sodium nitroprusside on relaxation could not be interpreted; new experiments to explore Imeglimin's effect on relaxation are in progress

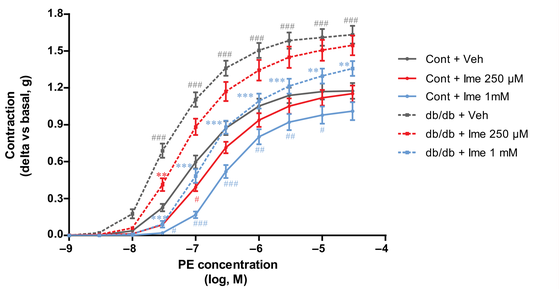

Figure 5. Imeglimin reduced the contractile response in a dose-dependent manner
Cont, control; Ime, Imeglimin; Veh, vehicle
$^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 vs control vehicle; $^{**}P$<0.01, $^{***}P$<0.001 vs db/db vehicle

Conclusion

- *In vivo*, 7 days of treatment with Imeglimin at the dose of 150 mg/kg prevents endothelial dysfunction observed in STZ-control mice after a week of exposure to hyperglycemia. This was shown with both PIV- and ACh-induced vasodilation
- *In vitro*, Imeglimin dose-dependently reduced PE-induced contractility of aortic rings from both normal and diabetic animals, restoring the normal contractility in aortic rings from diabetic animals at the highest concentration of 1 mM
- These preliminary *in vivo* and *in vitro* results on vascular reactivity suggest that Imeglimin may exert protective effects on diabetes-induced micro- and macrovascular complications

Conflicts of Interest

Pascale Fouqueray, Sophie Hallakou-Bozec and Sébastien Bolze are Poxel employees; Professor Harold Lebovitz is a member of Poxel scientific advisory board.

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