

Introduction

Type 1 diabetes (T1D) results from the progressive destruction of pancreatic β cells by the immune system, and clinically develops when β -cell mass drops to 10-15% of its initial value. Presently, the autoimmune character of T1D is challenged, yet it is indisputable that inflammation plays a key role in its development.

Hypothesis & Objectives

We hypothesized that glucotoxicity could contribute to β -cell mass destruction through maintenance of inflammation. Our aim is to evaluate empagliflozin (EMPA) potential to protect β -cell mass against glucotoxicity, and the potential of GABA to increase the residual β -cell mass after diagnosis of T1D.

Results

Figure 1. Glycemic evolution during treatment. The grey area corresponds to the streptozotocin (STZ) injection period (four days) and the rest period (three days). The bars represent the SEM. Number of mice per group is indicated ($n \geq 3$).

Figure 2. Effect of empagliflozin, GABA and their combination on the homeostasis of glucose. Empagliflozin and GABA treatment improve glucose tolerance of diabetic mice. IPGTT was performed at the end of treatment. Blood glucose was measured at different times after a 20% glucose injection in mice. The bars represent the SEM. Number of mice per group is indicated ($n \geq 3$). *, †, # = $p < 0.05$.

Figure 3. Effect of empagliflozin, GABA and their combination on free fatty acid levels. Empagliflozin and GABA treatment improve FFA level. Blood samples of mice were collected at different time points: at the end for FFA dosage. * = $p < 0.05$. The bars represent the SEM. Number of mice per group is indicated ($n \geq 3$).

Figure 4. Effect of empagliflozin, GABA and their combination on islet density and number. Empagliflozin and GABA treatment improve islet area/pancreas area ratio but not islet number/pancreas area ratio. The pancreas of mice were collected and its tail was used for hematoxylin and eosin coloration. The islets area and pancreas areas (A) and the islets number (B) were calculated using Leica software. a, b = $p < 0.05$. The bars represent the SEM. Number of mice per group is indicated ($n \geq 3$).

Figure 5. Effect of empagliflozin, GABA and their combination on pancreatic islet preservation. Empagliflozin and GABA treatment improve islet structure. Pancreas of mice was removed and its tail was used for insulin and glucagon staining (A-E). The red, green and blue staining corresponds to the insulin, glucagon and dapi (nucleus). Scale bar = 100 μ m. The percentage of regular and irregular islets was performed using Visiopharm software (F). a, b = $p < 0.05$. The bars represent the SEM. Number of mice per group is indicated ($n \geq 3$).

Figure 6. Effect of empagliflozin and GABA on the expression of ER stress, oxidative stress and inflammation markers. Empagliflozin and GABA treatment improve ER stress, oxidative stress and inflammation gene expression. At the end of the treatment, the pancreas of mice was taken and its body was used for RT-qPCR for the expression of ER stress (A), inflammation (B) and oxidative stress (C) markers. * = $p < 0.05$. The bars represent the SEM. Number of mice per group is indicated ($n \geq 3$).

Discussion & Conclusion

Empagliflozin and GABA, used in monotherapy, have positive effects on β -cell mass preservation or proliferation through an indirect effect on islet cell inflammation and ER stress. Further research is mandatory to evaluate whether empagliflozin and GABA may be translated to therapeutic protocols to protect β -cell mass after diagnosis of T1D .

Acknowledgments

We thank Caroline Bouzin and her team (Plateform 2IP), Nicolas Van Baren and his team (DDI) for their expert technical assistance. This study was supported by grants from the Belgian Society for Pediatric Endocrinology and Diabetology (BESPEED) and Fonds de Recherche Clinique (FRC) of UCLouvain.