Empagliflozin effect on the Central Nervous System (CNS): an *in vitro* study on primary neuronal cell cultures

Nikolaos P. Tzavellas[1,2], Athena S. Davri[1], Andreas P. Katsenos[1,2], Yannis V. Simos[1,2], Ilias P. Nikas[3], Chryssa Bekiari[4], Panagiotis Lekkas[1], Stavroula A. Paschou[5], Dimitrios Peschos[1,2], Spyridon Konitsiotis[6], Patra Vezyraki[1] & Konstantinos I. Tsamis[1,2]

[1]Laboratory of Physiology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece.
[2]Nanomedicine and Nanobiotechnology Research Group, University of Ioannina, Greece.
[3]School of Medicine, European University Cyprus, Nicosia, Cyprus.
[4]Laboratory of Anatomy, Histology & Embryology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece.
[5]School of Medicine, National and Kapodistrian University of Athens, Department of Clinical Therapeutics, Alexandra Hospital, Athens, Greece.
[6]Department of Neurology, University Hospital of Ioannina, Ioannina, Greece

INTRODUCTION

Sodium-glucose transporters (SGLTs) are protein channels that regulate glucose transport through the cell membrane, playing a central role in the reabsorption of glucose into the renal tubules (***Fig.1a***). SGLT2 and SGLT1 have been found in the cells of different organs as well as in the brain. Alzheimer's disease (AD) and type 2 diabetes mellitus (T2D) share common pathogenetic mechanisms, like oxidative stress and inflammation, supporting the rational of testing the therapeutic potential of antidiabetic drugs in the treatment or prevention of AD. Empagliflozin, an SGLT2 inhibitor, improves glycemic control in patients with T2D, reducing renal glucose reabsorption, and is a promising treatment option for neurodegenerative diseases (***Fig.1b***)[1-4].

AIMS

Metabolic neuronal pathways and their effect on the central nervous system (CNS) play a key role in neuronal degeneration and death. Considerable number of drugs modulating these pathways have been tested so far. Empagliflozin comprises a promising novel therapeutic approach against neurodegenerative disorders and the present study aims to test its direct effect on neurons, since the possibility of neurotoxicity through glucose and energy deprivation has never been addressed before.

MATERIALS AND METHODS

Primary neuronal cell cultures were developed from wild type Sprague Dawley rats and neurons were exposed to different concentrations of empagliflozin for 48 hours, during either their early development in the first week, or after two weeks *in vitro*. Neuronal survival was evaluated by MTT assay and development of the dendritic tree was assessed under fluorescent microscopy after transfection with GFP or stain with DiI. A morphometric comparative study was also performed with the ImageJ software and NeuronJ plug-in.

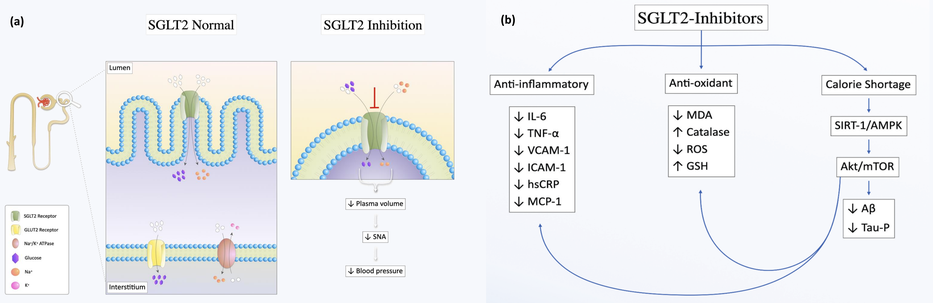


Fig.1 (a) *The effect of* SGLT2i on kidney. ***(b)*** The cellular effects of SGLT2i.

RESULTS

The MTT assay illustrates that empagliflozin in concentrations between 0,001μM and 8μM does not affect cell viability in primary hippocampal neurons. However, neurons seem to exhibit toxic consequences in greater concentrations of empagliflozin ***(Fig.2)***. Evaluation of the neuronal dendritic tree after 48h treatment with 10μM empagliflozin didn't reveal morphologic alterations ***(Fig.3)***. Morphometric analysis showed slight reduction in the total dendritic length; however, the result didn't reach statistical significance ***(Fig.4)***.

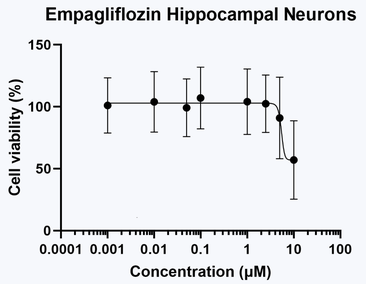


Fig.2 MTT assay**.** Empagliflozin at concentrations between 0,001μM and 8μM did not significantly alter neuronal survival. Concentrations above 8μM seem to exert toxic effects on primary hippocampal neurons.

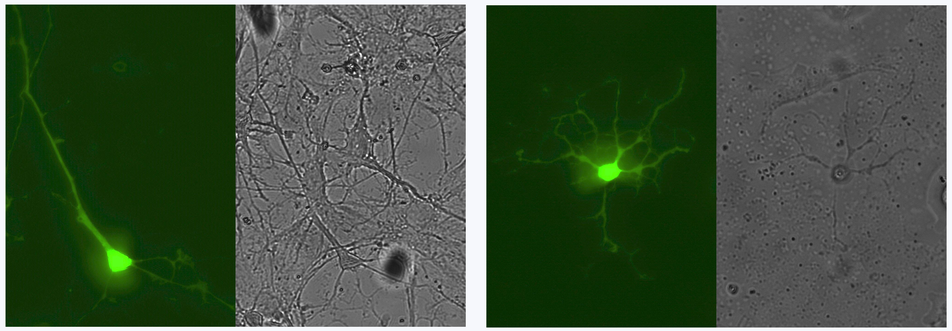
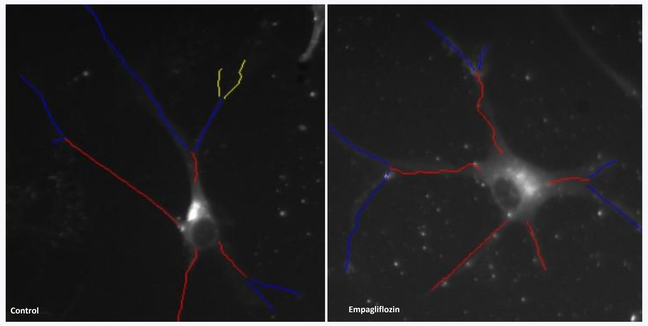


Fig.3 Morphologic evaluation of the dendritic arborization on pyramidal-like and non-pyramidal neurons after GFP transfection and empagliflozin treatment (phase contrast and GFP immunofluorescence).



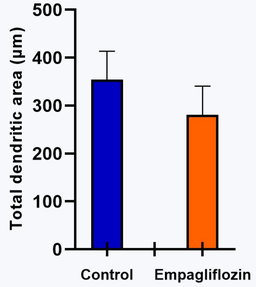


Fig.4 Morphometric analysis of dendrites in primary hippocampal neuron culture, with ImageJ software and NeuronJ plug-in, revealed a slight reduction in the total length of the dendritic tree after exposure for 48h to 10μM empagliflozin compared to control neurons. However, the result didn't reach statistical significance.

CONCLUSION

Our results show that low and medium concentrations of empagliflozin on neurons do not exert any toxic effect. However, higher concentrations of empagliflozin in the CNS may cause neurotoxicity, in terms of glucose deprivation. Its suitability as a new neuroprotective agent should be investigated with further *in vitro* and *in vivo* testing.

REFERENCES

1. Andreas P. Katsenos *et al*. New treatment approaches for Alzheimer's disease: preclinical studies and clinical trials centered on antidiabetic drugs, Expert Opinion on Investigational Drugs, 31:1 (2022)
2. Pawlos, A., Broncel, M., Woźniak, E. & Gorzelak-Pabiś, P. Neuroprotective Effect of SGLT2 Inhibitors. *Molecules* **26**, 7213 (2021).
3. Tuttle, K. R. *et al.* SGLT2 Inhibition for CKD and Cardiovascular Disease in Type 2 Diabetes: Report of a Scientific Workshop Sponsored by the National Kidney Foundation. *Diabetes* **70**, 1–16 (2021)
4. Hierro-Bujalance C. *et al.* Empagliflozin reduces vascular damage and cognitive impairment in a mixed murine model of Alzheimer's disease and type 2 diabetes. Alzheimers Res Ther. 2020 Apr 7;12(1):40.

SCAN ME