Protective effect of Empagliflozin on αKlotho/Autophagy protein LC3 in diabetic retinopathy: Evidence from diabetic mice model

Nakhoul Nakhoul[1,2], Ertracht Offir[3], Igbariye Anas[4], Irit Mann[5], Hagar Tadmor[6], Ranin Saed[2,6], Nakhoul Farid[2,3] Atar Shaul[2,3,7]

[1]The Ophtalmology Division, Baruch Padeh Medical Center, Poriya, [2]Azrieli Faculty of Medicine, Safed, Bar-Ilan University, [3]Cardiovascular Laboratory, Medical Research Institute, Galilee Medical Center Nahariya, [4]Rambam Medical Center, Haifa, [5]Rappaport Institute, Faculty of Medicine, Israel Institute of Technology – The Technion, Haifa, [6]Diabetes and Metabolism Lab, Baruch Padeh Poriya Medical Center, [7]The Cardiology Department, Galilee Medical Center, Nahariya, Israel.

Background

Diabetic retinopathy (DR) is a leading cause of blindness, and its incidence is increasing worldwide. The serious proliferative DR type is characterized by an abnormal growth of small blood vessels in the eye due to hypoxia and synthesis of vascular endothelial growth factor (VEGF) disc neo-vascularization, vitreous hemorrhages, retinal detachment and blindness. Hyperglycemia triggers pro-inflammatory mediators, which activate oxidative stress and inflammatory signal. Beyond glycemic control, most DR therapies are effectuated late, when vision is already incurable. The proteins αKL and LC3 are involved in the protective pathway of autophagy. Both abundant in renal and retinas tissue, thus, may used as markers for autophagy.

Empagliflozin (EMPA) is a sodium-glucose cotransporter 2 inhibitors (SGLT2i) a anti-diabetes drug, which function at the kidney to inhibit glucose re-absorption. Lately, additional efficacies were attributed to this family of drugs.

Hypothesis

We hypothesize that αKlotho/Autophagy protein LC3 are involved in the DR progression and subsequent eye deterioration, and that EMPA will attenuate DR deleterious markers.

Material & Methods

BTBR mice with the ob/ob leptin-deficiency mutation that develops spontaneously severe T2DM and C57/BL mice (control) were used. EMPA was administrated to the T2DM mice via drinking water for 12 weeks. Finally, mice retinas were removed and subjected to further histological analysis: Immunohistochemistry and Immunofluorescence staining for $\alpha KL_{LC3,protein\ expression\ level.}$

Results

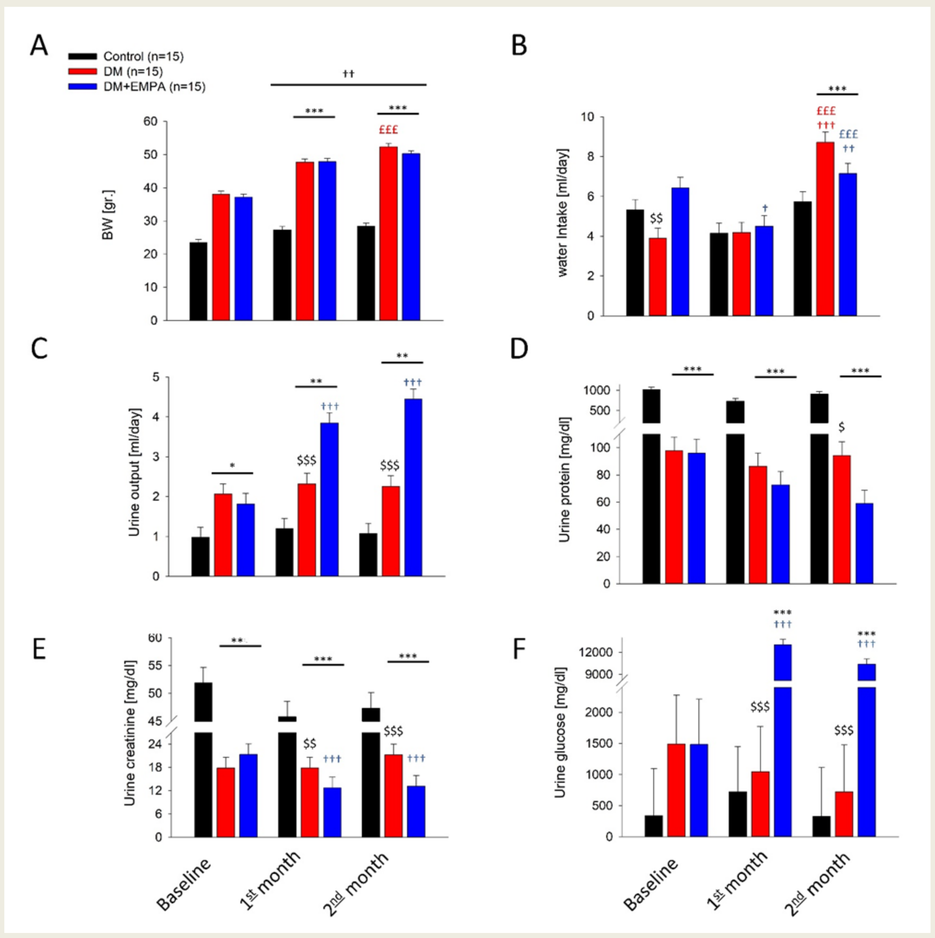

Figure1: Mice physiological parameters. A. mean weight, B. water intake, C. urine output, D. Urine protein exertion, E. Urine creatinine, F. Urine glucose level. * *vs.* C57/Bl, $ *vs.* DM+EMPA, £££ *vs.* one month, † *vs.* BL.

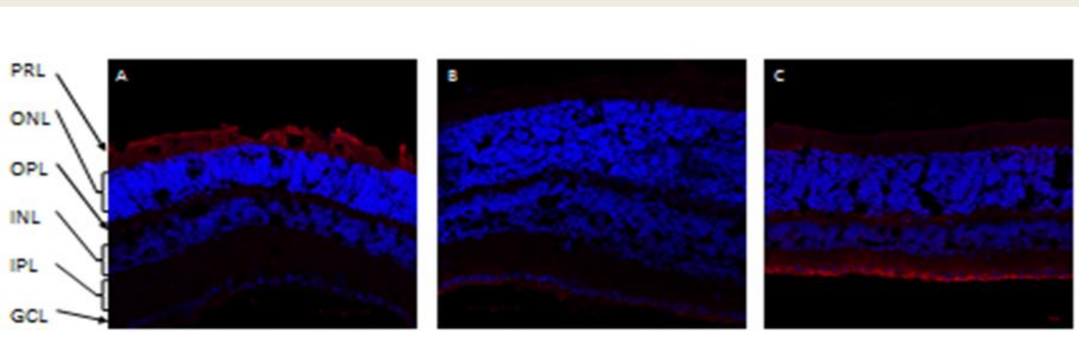

Figure2: representative immunofluorescence of αKL expression in retinal ganglionic cell layer (GCL), of A. C57/Bl mice B. DM mice. C. DM+EMPA mice. Red αKL positive pixels, Blue- DAPI (nuclei).

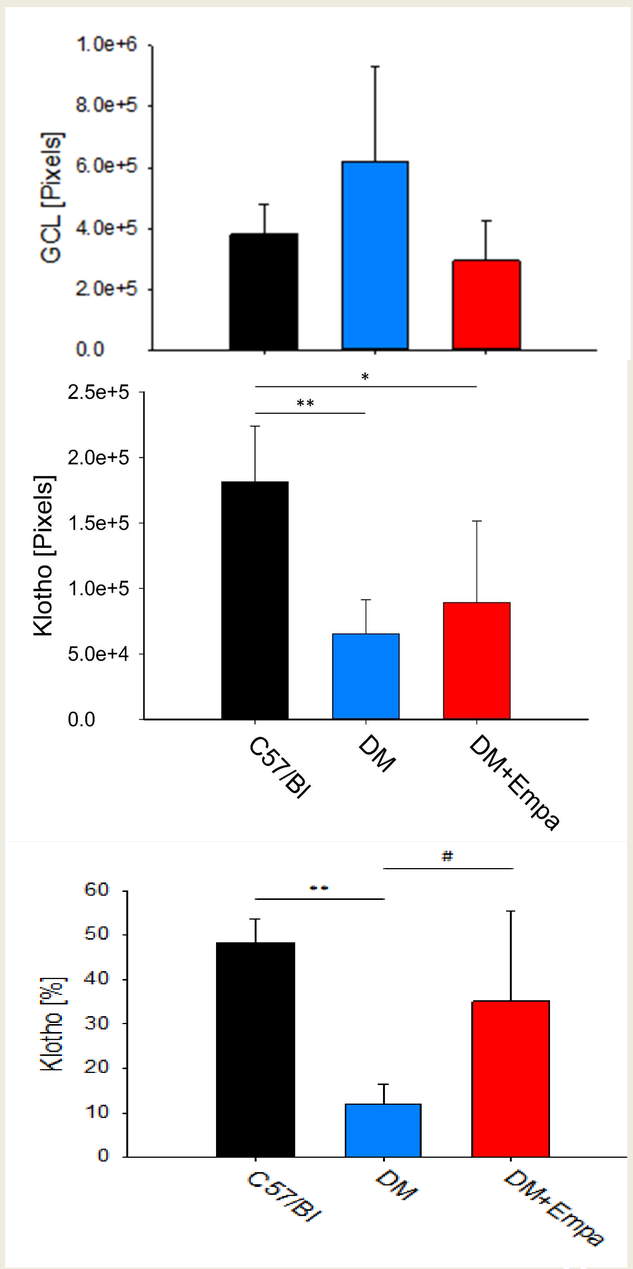

Figure 3: ganglionic cell layer (GCL) width. GCL width was comparable among groups. Results are shown as mean ± SEM.

Figure 4: Klotho (number of pixels) in the GCL. There was significant reduction in the pixels number in T2DM mice *vs.* control (P<0.05). EMPA treatment decreased less the Klotho expression (P<0.01).

Figure 5: Klotho (area staining %) in the GCL. There was significant decrease in Klotho area in the DM group *vs.* control (P<0.01). EMPA reversed Klotho expression (P<0.05 *vs.* DM)

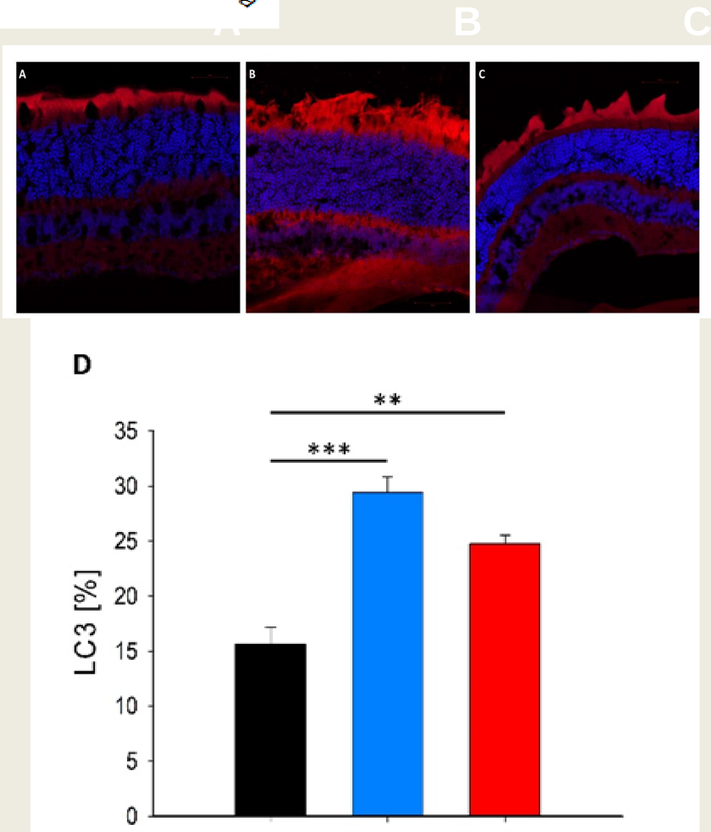

Figure 6: LC3 expression % in GCL retina in the three experimental groups. A. representative photographs taken from a C57/Bl mice, B. DM mice, C. DM+EMPA mice. D. summary of measured LC3 expression, presented in % of tissue area.

Discussion & Conclusion

1. Our data suggested that αKL protein and the autophagy proteins LC3 are involved in the pathogenesis of DR. We suggested that αKL and the autophagy key protein LC3 modulators could probably be potential protective factors against retinopathy develops in T2DM patients.
2. The anti-aging protein αKL plays a critical role in retinal ganglionic cells function, especially the GCL. Our study revealed a significant decrease in retinal αKL protein expression in T2DM mice model, and restored by the new anti-diabetic EMPA treatment.
3. It appears that Klotho may be involved in several physiological processes such as chronic hyperglycemia in T2DM vascular complications.