Monocyte and endothelial dysfunction induced by hyperglycemia is reversed by Empagliflozin mainly through glucose-transport independent mechanisms

D. Semo¹, J. Obergassel¹, M. Dorenkamp¹, R. Godfrey¹, J. Waltenberger²

¹University Hospital Münster, Department of Cardiology I – Coronary and Peripheral Vascular Disease, Heart Failure, Münster, Germany; ²University of Münster, Department of Cardiovascular Medicine, Medical Faculty, Münster, Germany

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Type II Diabetes mellitus (T2DM) leads via hyperglycemia (HG)-associated inflammation and oxidative stress to vascular cell dysfunction. The EMPA-REG trial revealed a reduction of cardiovascular mortality in T2DM patients by Empagliflozin, a sodium-glucose co-transporter-2 (SGLT-2) inhibitor. SGLT-2 is expressed on monocytes and endothelial cells (EC), therefore the aim of this study was to investigate the potential role of Empagliflozin for glucose transport regulation and improvement of HG-induced dysfunction of monocytes and EC.

HUVEC and HCAEC were used as EC model cells. Primary human monocytes were isolated from T2DM patients and healthy controls. The cells were exposed to HG conditions in vitro in presence of 40 or 100 ng/ml Empagliflozin. Using RT-qPCR and FACS, expression levels of SGLT-2 were analysed. Glucose uptake assays were performed with a fluorescent derivative of glucose, 2-NBDG. To measure reactive oxygen species (ROS) accumulation, we used the H2DFFDA method via FACS and fluorescence microscopy. Monocyte and EC chemotaxis was analysed using modified Boyden chamber assays.

We could prove an expression of SGLT-2 in primary human monocytes and EC. The transcripts of SGLT-2 in T2DM monocytes did not show a difference. SGLT2-levels in monocytes and EC were not altered by HG. In presence of GLUT inhibitor in vitro glucose uptake assays revealed the ability of SGLT-2 inhibition to mildly suppress the glucose uptake, whereas uptake by monocytes and EC remained comparable. Nevertheless, in presence of Empagliflozin HG-induced ROS accumulation in monocytes (1.3-fold, p=0.021) and ECs (1.5-fold, p=0.003) was reduced. HG monocytes and EC readily exhibited an impaired chemotaxis behaviour. Treating the cells in HG with Empagliflozin reversed the PI GF-1 resistance phenotype of HG monocytes (1.4-fold, p=0.002). Comparably, Empagliflozin was able to rescued the blunted VEGF-A responses of HG ECs (1.35-fold, p=0.028), which could be ascribed to restoration of VEGFR-2 receptor levels on the EC surface. Most of the aberrant phenotypes exhibited by HG monocytes and EC were entirely recapitulated by induction of oxidative stress. We could mimic these effects of Empagliflozin using the general antioxidant N-acetyl-L-cysteine (NAC).

Our study reveals a beneficial role of Empagliflozin for reversing HG-induced vascular cell dysfunction. The expressed SGLT-2 on monocytes and EC does not serve as primary glucose transporter. So it is highly to speculate that HG-mediated enhanced glucotoxicity in those cells is not prevented by Empagliflozin through inhibition of glucose uptake itself. Reduction of oxidative stress by Empagliflozin was revealed as central mechanism for an improved function of HG monocytes and EC in vitro. Finally, we conclude that antioxidant properties of Empagliflozin reverse vascular cell dysfunction independently of glucose transport. This is likely to support the beneficial effect of Empagliflozin in cardiovascular disease.