SGLT2 independent effects of the SGLT2 inhibitor empagliflozin

Focus on the heart

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Chapter 06

SGLT2 inhibitor Empagliflozin reduces infarct size independent of SGLT2

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Empagliflozin, a sodium glucose cotransporter-2 inhibitor (SGLT2i), developed to facilitate glycosuria in diabetic patients, has a large beneficial effect in patients with heart failure. Empagliflozin has SGLT2 dependent effects on kidney (modulating fluid balance, metabolism and hemodynamics) but early preclinical research has also demonstrated off target effects of SGLT2i on cells largely devoid of SGLT2.[1,2] Consequently, ambiguity exists to what extent the beneficial effects of SGLT2i's are dependent on SGLT2 or not. Because SGLT2i's cause a reduction in infarct size[3], it is proposed that the drugs have beneficial cardiovascular effects in acute cardiac ischemia-reperfusion injury (IRI).[4] In a multicenter observational registry study of diabetic AMI patients, it was shown that patients receiving SGLT2i's exhibited reduced inflammation and infarct size compared to patients receiving other glucose-lowering agents.[5] Previous preclinical work has shown that possibly the activation of STAT3, PI3K and increases in fibroblast growth factor-2 and caveolin-3 expression mediate the AMI protective effects of SGLT2i's.[3] We hypothesize that these effects are independent of the presence of the SGLT2 protein and therefore represent an off-target cardiac mechanism.

We generated a somatic *Slc5a2* (encoding SGLT2) deficient mouse model on a C57Bl/6N background. All procedures involving animals were approved by institutional and national authorities. *Slc5a2* knock-out mice were generated using CRISPR/Cas9 and 2 sgRNAs targeting TCGTGGTGCTGCTCCTCGGA and CGGACAGGTAGAGGCGAATA in exon 4 of *Slc5a2*, cloned into BsaI (NEB) linearized DR274 vector (Addgene #42250). The sgRNAs and Cas9 mRNA were injected into the cytoplasm of one-cell embryos, and founders were screened by PCR with the primers CAGGCACTTCCGCTGTGTCT (Forward (FW) and ACTTCACTGGGTGGTTCTACCTT (Reverse(RV). q-RT PCR was performed with primers (FW: 5′-GGATGGCTTTTTGTGCCAGTG-3′ and RV:5′-CAAAGCGCTTGCGGAGGTA-3′).

Slc5a2/SGLT2 deficiency was confirmed at the DNA, mRNA and protein level in the kidney (Fig A-C). No cardiac SGLT2 was detected in WT or KO (Fig C). At 10-13 weeks of age, body and kidney weights were unaltered by SGLT2 deficiency (Fig. D-E). The SGLT2 KO was functionally confirmed by high glucose in the urine, whereas plasma levels of glucose and ketones in the non-fasted, anesthetized condition were unaffected (Fig. F-H). Thus, SGLT2 KO was successfully established on a C57Bl/6N background and was confirmed by increased urinary glucose.

We then addressed the question whether the global chronic ablation of SGLT2 would affect the intrinsic sensitivity of the heart to ischemia-reperfusion injury (IRI) by using the isolated Langendorff-perfused mouse heart model. After 20 min stabilization, hearts were subjected to 30 min global ischemia followed by 90 min reperfusion. At baseline, coronary flow, developed systolic left ventricular pressure (DLVP) and heart rate were not significantly different between WT and SGLT2 KO (Fig. I-K), demonstrating that the global deletion of SGLT2 does not affect intrinsic

cardiac performance parameters. Following I/R, end-diastolic left ventricular pressure rose from the preset levels of 3-7 mmHg to 50.7 ± 6.1 mmHg, DLVP declined to 34.8 ± 4.5 mmHg and heart rate to 309 ± 12 beats/min for WT hearts. These values were unaltered for KO hearts (Fig. L-N). Finally, also infarct size (Fig. O) was not different between the genotypes. Overall, the *ex vivo* data demonstrate that chronic deletion of whole-body SGLT2 does not alter the intrinsic sensitivity of the heart to I/R injury.

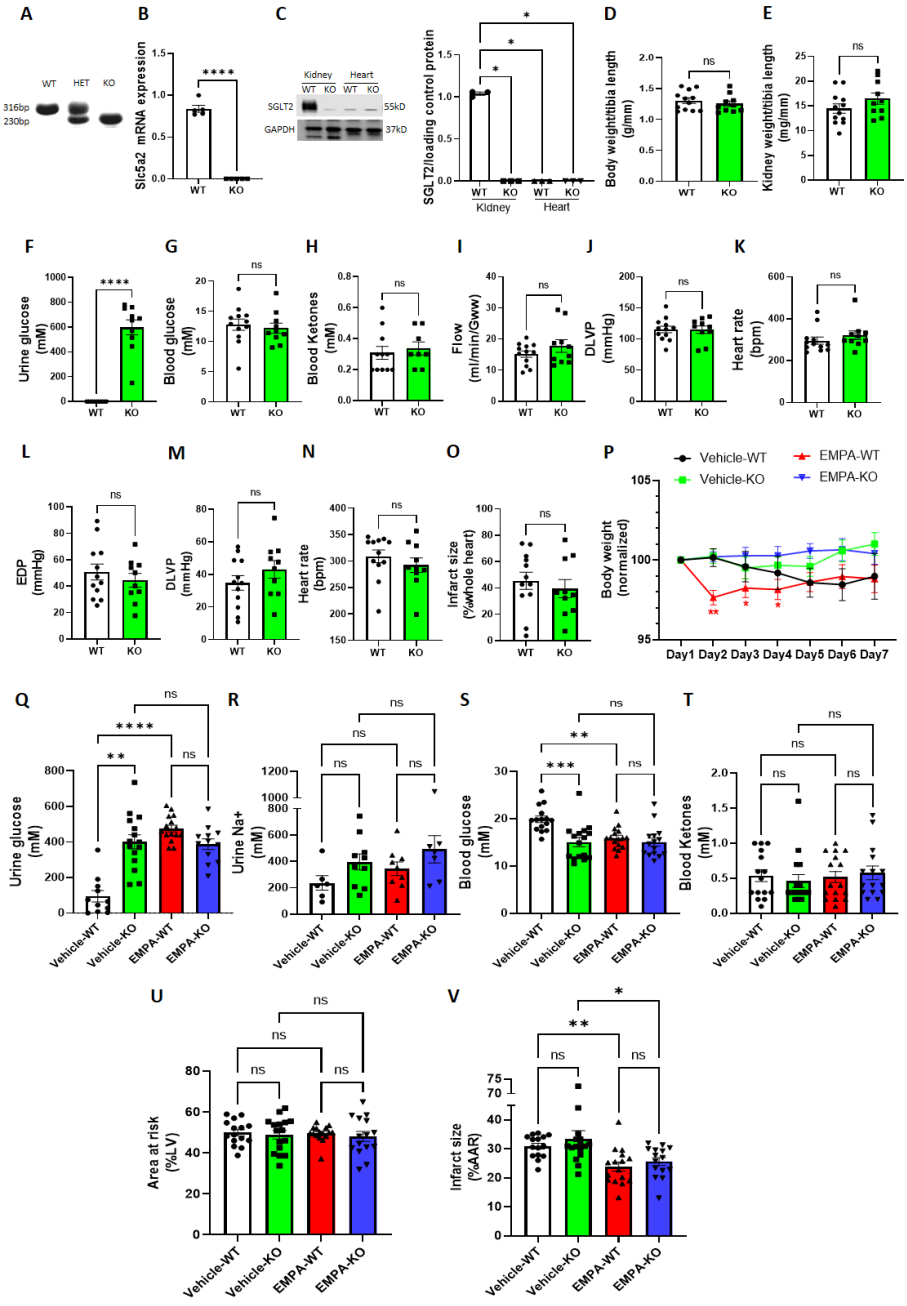
Next, we determined whether the protection by Empagliflozin (EMPA) against *in vivo* I/R injury[3] persisted in the absence of the SGLT2 protein. WT and SGLT2 KO animals were 7 days pretreated by oral gavage with vehicle (DMSO) or EMPA (10 mg/kg/day) prior to 30 min LAD occlusion and 2 h reperfusion. Empagliflozin treatment reduced body weight up to day 4 after start of gavage in WT but not in SGLT2 KO mice and not at later stages, indicating that the short term effect of EMPA on BW was mediated through SGLT2 inhibition (Fig. P). Urine glucose determined at excision heart was equally elevated in vehicle-KO, EMPA-WT and EMPA-KO in comparison to vehicle-WT, confirming that urine glucose is largely dependent on impaired SGLT2 activity (Fig. Q). Urine sodium at excision heart was not significantly different between groups, although there was a trend for increased urine sodium in the vehicle-KO and EMPA-treated animals (Fig. R). Overall, infarct protection by EMPA is not related to factors determining urine glucose or sodium such as SGLT2 inhibition/deletion. Plasma glucose just before start of ischemia was decreased in vehicle-KO and EMPA-treated animals in comparison to vehicle-WT (Fig S). Similarly, EMPA did not raise ketone levels in WT or KO animals (Fig T).

Area at risk was similar for all four groups (Fig U). EMPA pretreatment caused a reduction of infarct size in both WT and KO-groups in comparison to both vehicle treated groups, and the chronic deletion of SGLT2 in itself did not reduce the *in vivo* infarct size (Fig. V). Infarct size was similar between males and females within each group (data not shown).

These data show 1) that whole body removal of SGLT2 alone does not mediate the beneficial cardiovascular effects associated with pharmacological SGLT2-inhibition, and 2) that EMPA causes a reduction in infarct size, independent of the presence of the SGLT2 protein and that this effect cannot be explained by changes in blood glucose or ketone levels.

In summary, we herein report and validate a new global SGLT2 knockout mouse on a C57Bl/6N background and demonstrated that beneficial cardiovascular effects of EMPA are independent of SGLT2. Although heart failure was not studied, the similarities in the underlying causal mechanisms of heart failure and I/R injury, i.e. ionic disturbances, inflammation and oxidative stress, at least suggest that the current findings that SGLT2i's cardioprotective effects against cardiac infarction are independent of SGLT2 may also apply to HF pathologies. Further studies are now

warranted to test for SGLT2 dependency on EMPA's beneficial effects in heart failure models



Legend

Figure SGLT2 deletion does not protect the isolated heart against ischemia-reperfusion injury (IRI), and cardioprotection by Empagliflozin in vivo is independent of SGLT2 protein. All procedures involving animals were approved by institutional and national authorities. All data is available from the authors upon reasonable request. Statistical analysis was performed using GraphPad Prism v9. Results are presented as mean±SEM. Data normality was examined using the Shapiro-Wilk test (with α=0.05) followed by parametric or non-parametric testing accordingly. **A**, PCR product was sequenced to screen for deletions that will cause a frame shift of the *Slc5a2* transcript. **B**, *Slc5a2* mRNA loss in kidney was confirmed by qRT-PCR; unpaired *t* test (2-tailed)**. C**, SGLT2 protein in kidney and heart (AB: Proteintech, 24654-1-AP); Kruskal-Wallis test with Dunn's multiple comparisons. **D-N**, Langendorff-experiments using 11-13 wks old littermate mice (n=12 for WT (6 male, 6 female); n=10 for KO (6 female, 4 male)). **D-E**, Body and kidney weight to tibia length; unpaired *t* test (2-sided). **F**, urine glucose before anesthesia; Mann-Whitney test. **G-H**, tail venous blood glucose (unpaired 2-sided *t* test) and ketone levels (Mann-Whitney test) in pentobarbital (95 mg/kg) anesthetized animals before surgery. **I-K**, baseline cardiac performance (Langendorff-perfused mouse hearts, perfused at constant flow at an initial baseline perfusion pressure of 80 mm Hg with Krebs-Henseleit buffer containing 1% albumin-0.2 mM palmitate, 5.5 mM glucose and 0.5 mM glutamine; a water-filled balloon was inserted for left ventricular pressure monitoring and diastolic ventricular pressure set between 3-7 mmHg). **I**, coronary flow at baseline; Mann-Whitney test. **J**, developed left ventricular pressure (DLVP) at baseline; unpaired 2-sided *t* test. **K**, heart rate at baseline, Mann-Whitney test. **L-O**, cardiac function recovery and infarct size after 30 min global ischemia and 90 min reperfusion. Post-experimentally, infarct size was determined by planimetry using TTC staining by a researcher blinded to group allocation. **L**, end-reperfusion end-diastolic pressure (EDP); unpaired 2-sided *t* test. **M**, DLVP; unpaired 2-sided *t* test. **N**, heart rate; Mann-Whitney test. **O**, infarct size relative to whole heart (%IS); unpaired 2-sided *t* test. **P-V**, EMPA pretreatment and in vivo cardiac I/R using 10-18 wks old SGLT2 KO and WT littermate mice. **P**, body weight, normalized to day 1, of SGLT2 KO and WT littermates during the 7 days treatment with EMPA (10 mg/kg/d) or vehicle (dimethyl sulfoxide, 6 µl/g) by oral gavage, vehicle+WT (n=15; 7 female/8 male), vehicle+KO (n=16; 8 female/8 male), EMPA+WT (n=16, 7 female/9 male) and EMPA+KO (n=15; 8 female/7 male). Vehicle-WT and EMPA-KO: One-way ANOVA with Bonferroni's multiple comparisons test; Vehicle-KO and EMPA-WT: Kruskal-Wallis test with Dunn's multiple comparisons. On day 8 one additional vehicle or EMPA dose was delivered orally, animals anesthetized (ketamine (100 mg/kg), xylazine (20 mg/kg) and atropine (0.6 mg/kg)) and ventilated, tail vein blood collected just before start ischemia for glucose and ketone measurements, LAD occluded for 30 min followed by 2 h reperfusion, urine collected, heart excised and cannulated for Evans Blue perfusion and TTC staining for determination of % area at risk and % infarct size, respectively. **Q-R**, urine glucose and sodium at end experiment for all 4 groups; Kruskal-Wallis test with Dunn's multiple comparisons and one-way ANOVA with Bonferroni's multiple

comparisons test, respectively. **S-T**, blood glucose and ketone for all 4 groups; Kruskal-Wallis test with Dunn's multiple comparisons. **U-V**, % area at risk (AAR) of left ventricle and % infarct size of AAR in I/R animals; Kruskal-Wallis test with Dunn's multiple comparisons.

References

1. Baartscheer A, Schumacher CA, Wüst RCI, Fiolet JWT, Stienen GJM, Coronel R, Zuurbier CJ. Empagliflozin decreases myocardial cytoplasmic Na^+ through inhibition of the cardiac Na^+/H^+ exchanger in rats and rabbits. *Diabetologia*. 2017;60:568-573. doi: 10.1007/s00125-016-4134-x

2. Chen S, Coronel R, Hollmann MW, Weber NC, Zuurbier CJ. Direct cardiac effects of SGLT2 inhibitors. *Cardiovasc Diabetol*. 2022;21:45. doi:10.1186/s12933-022-01480-1

3. Nikolaou PE, Mylonas N, Makridakis M, Makrecka-Kuka M, Iliou A, Zerikiotis S, Efentakis P, Kampoukos S, Kostomitsopoulos N, Vilskerts R, et al. Cardioprotection by selective SGLT2-inhibitors in non-diabetic mouse model of myocardial ischemia/reperfusion injury: a class or a drug effect? *Bas Res Cardiol*. 2022;117:27. doi: 10.1007/s00395-022-00934-7

4. Udell JA, Schuyler Jones W, Petrie MC, Harrington J, Anker SD, Bhatt DL, Hernandez AF, Butler J. Sodium glucose cotransporter-2 inhibition for acute myocardial infarction. *J Am Coll Cardiol*. 2022;79:2058-2068. doi: 10.1016/j.jacc.2022.03.353

5. Paolissa P, Bergamaschi L, Santulli G, Gallinoro E, Gragnano F, Sardu C, MIleva N, Foa A, Armillotta M, Sansonetti A, et al. Infarct size, inflammatory burden, and admission hyperglycemia in diabetic patients with acute myocardial infarction treated with SGLT2-inhibitors: a multicenter international registry. *Cardiovasc Diabetol*. 2022;21:77. Doi:10.1186/s12933-022-01506-8.