DAPAGLIFLOZIN AMELIORATES ISCHEMIA REPERFUSION RENAL INJURY VIA HIF1 INDUCTION.

Dae Eun choi1, Hyunsu Choi2, Jin Young Jeong1, Yoon-Kyung Chang3, Sarah Chung1, Ki-Ryang Na1, Kang Wook Lee1

1chungnam national university hospital, nephrology, daejeon, KOREA, REPUBLIC OF, 2Daejeon Saint Mary Hospital, Clinical Research Institute, daejeon, KOREA, REPUBLIC OF, 3Daejeon Saint Mary Hospital, Nephrology, daejeon, KOREA, REPUBLIC OF.

INTRODUCTION:

Dapagliflozin recently used for diabetes control. It inhibit sodium-glucose co-transporter type (SGLT) 2, which wastes the glucose to urine. Although SGLT2 KO mice study showed no reduction of inflammation markers in type 1 DM mice model, some studies showed SGLT2 inhibition have renal protection(reduce hyperfiltration and tubular oxidative stress) in Type1 DM. We evaluate whether SGLT2 inhibitor reduces the renal damage via ischemia reperfusion (IR). Also, we investigate the associating molecular pathway.

METHODS:

in vitro, IR was simulated by mineral oil in HK-2 cells. Cell survival, apoptosis signal pathway, reactive oxygen species (ROS) generation, HIF1, ERK, AMPK, PGC1 alpha were evaluated in control and IR HK-2 cell with or without SGLT2 inhibitor. In vivo 10 weeks C57BL/6 mice were divided into 4 groups; vehicle (n=5) and dapagliflozin (10mg/kg PO 4hr and 1hr before operation) treated sham group (n=5), vehicle n=7) and dapagliflozin (n=7) with IR (reperfusion 27 minutes after clamping of both renal artery and vein) renal injury. Kidneys and blood were harvested 24hr after IR injury. We performed real time RT-PCR, western blot and immunohistochemistry for molecular study and H&E stain and PAS stain for histologic examination.

RESULTS :

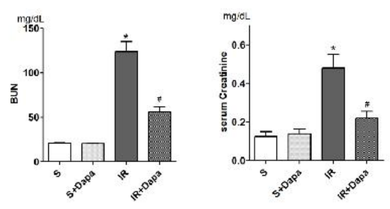

Figure 1. . Effects of dapagliflozin on renal function (S, sham; S + Dapa, sham + dapagliflozin; IR; vehicle-treated renal IR mice; IR + Dapa, dapagliflozin-treated IR mice). Dapagliflozin pretreatment reduced serum creatinine in IR-injured mice; n = 5–7 in each group. *P < 0.05 vs. vehicle-treated sham, #P < 0.05 vs. vehicle-treated IR. Bar represents mean ± s.d.(Standard deviation)

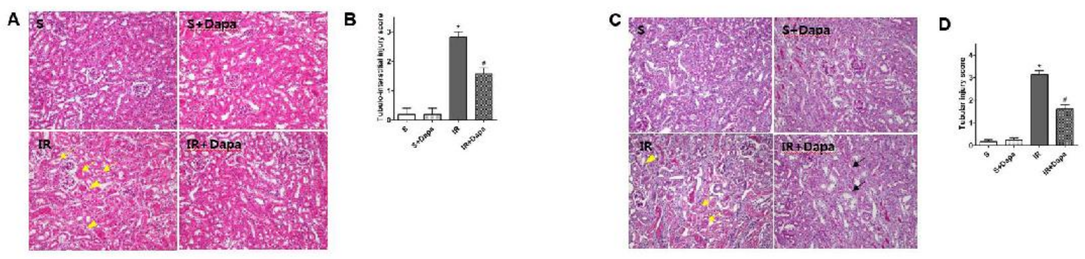

Figure 2 Representative kidney section stained for hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). (A) H&E stain, yellow arrows indicate cell debri and tubular necrosis. Yellow arrowheads indicate inflammatory cells. Original magnification, 200×. (B) Semi-quantitative analysis of tubule interstitial injury in wild-type and dapagliflozin- and/or albendazole-treated mice 24 h after renal IR injury (C) PAS stain. Yellow arrows indicate necrotized tubules or cast formation. Yellow arrowheads indicate loss of brush border or dilated tubules. Black arrow indicates brush border. Original magnification, 200×. (D) Semi-quantitative analysis of tubular injury in wild-type and dapagliflozin- and/or albendazole-treated mice 24 h after renal IR injury. *P < 0.05 vs. vehicle-treated sham, #P < 0.05 vs. vehicle-treated IR. Bar represents mean ± s.d.

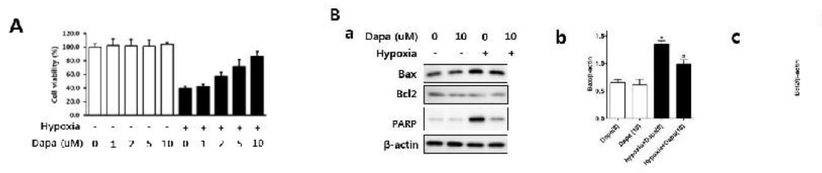

Figure 3. Effects of dapagliflozin on HK2 cell survival and apoptosis. (A) Dapagliflozi e-dependent manner. (B) Dapagliflozin decreased Bax and PARP expression in hypoxic HK2 cells. Bcl2 expression was unaffected by dapagliflozin in hypoxic HK2 c ntrol hypoxic HK2 cells. Bar represents mean ± s.d.

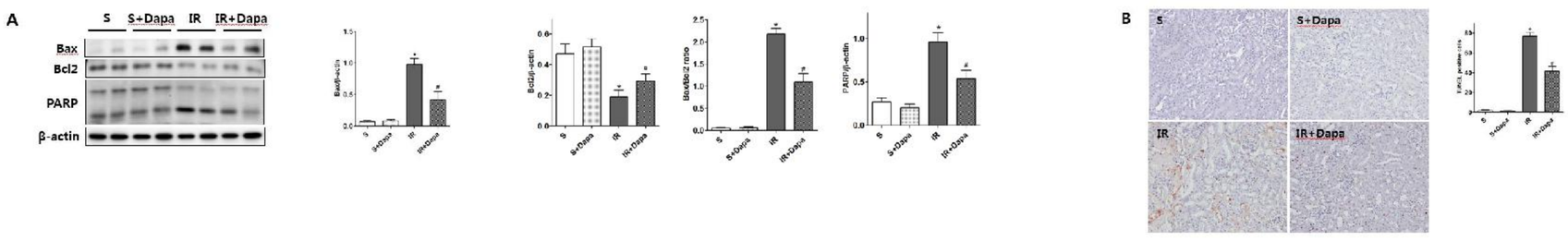

Figure 4. Effects of dapagliflozin on apoptosis in IR-injured kidneys. (A) Western blot analysis shows dapagliflozin decreased Bax expression and increased Bcl2 expression in IR-injured kidneys. Dapalgliflozin decreased PARP expression in IR-injured kidneys. (B) Representative kidney section. Dapagliflozin decreased the TUNEL-positive cells in an IR-injured kidney. *P < 0.05 vs. sham kidney, #P < 0.05 vs. IR kidney. Bar represents mean ± s.d.

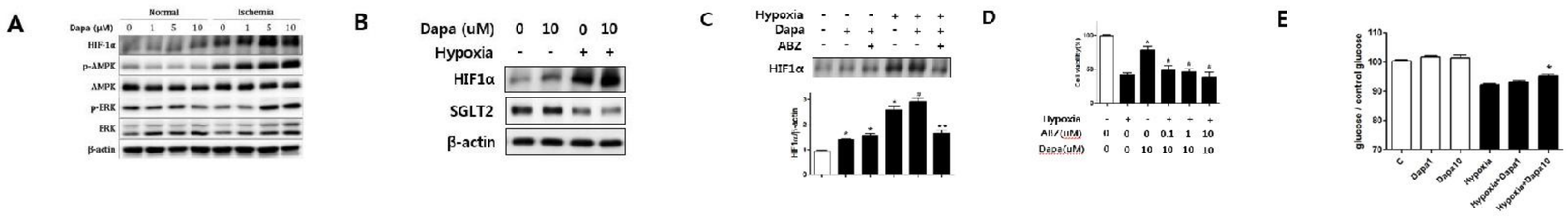

Figure 5. . Effects of dapagliflozin on HIF1 in HK2 cells. (A) Representative Western blot: dapagliflozin increased HIF1 expression and phosphorylation of AMPK and ERK in hypoxic HK2 cells. *P < 0.05 vs., dapagliflozin nontreated normoxic HK2 cells, #P < 0.05 vs. normoxic HK2 cells. **P < 0.05 vs. control hypoxic HK2 cells. (B) Representative Western blot: hypoxia increased HIF1 expression and decreased SGTL2 expression. Dapagliflozin pretreatment decrease SGLT2 expression in hypoxic HK2 cells. *P < 0.05 vs., dapagliflozin nontreated normoxic HK2 cells, #P < 0.05 vs. normoxic HK2 cells. **P < 0.05 vs. control hypoxic HK2 cells. (C) Dapaglilfozin pretreatment increase HIF1 expression in normoxic and hypoxic HK2 cells compared to control and hypoxic control HK2 cells respectively. Albendazole decreased HIF1 expression in dapagliflozin-treated hypoxic HK2 cells. *P < 0.05 vs., control HK2 cells, #P < 0.05 vs.control hypoxic HK2 cells. **P < 0.05 vs. albendazole nontreated - dapagliflozin treated hypoxic HK2 cells. (D) Albendazole decreased cell survival in dapagliflozin-treated hypoxic HK2 cells. *P < 0.05 vs., control hypoxic HK2 cells #P < 0.05 vs.dapagliflozin treated hypoxic HK2 cells. (E) Dapagliflozin decreased glucose uptake in hypoxic HK2 cells. *P < 0.05 vs., hypoxic HK2 cells. Bar represents mean ± s.d.

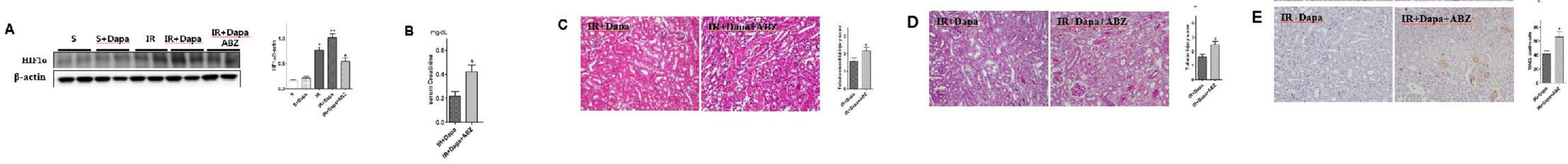

Figure 6 Effects of dapagliflozin on HIF1 in IR-injured kidneys (S, sham; S+Dapa, sham+dapagliflozin; IR; vehicle-treated renal IR mice; IR+Dapa, dapagliflozin-treated IR mice; IR+Dapa+ABZ, albendazole- and dapagliflozin-treated IR mice, n = 5–7 in each group). (A) Representative Western blot: dapagliflozin increased HIF1 expression in IR-injured kidneys. Albendazole decreased HIF1 expression in dapagliflozin-treated IR-injured kidney. (B) Representative Immunohistochemistry of HIF1. Dapagliflozin increase the HIF1 stained area in tubular area of IR kidney. Original magnification, 200× (C) The effects of dapagliflozin on renal function. Albendazole treatment elevated BUN and serum creatinine in dapagliflozin-treated IR mice. (D–F) Representative kidney section: albendazole treatment increase tubulointerstitial injury and TUNEL-positive cells in IR-injured kidneys. *P < 0.05 vs. vehicle-treated sham, **P < 0.05 vs. vehicle-treated IR, #P < 0.05 vs. dapagliflozin-treated IR. Bar represents mean ± s.d.

CONCLUSION :

dapagliflozin significantly increases HIF1 in IR injured kidney. Also it attenuates ischemia reperfusion renal injury.

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