Sirt1 activation protects HHE-induced oxidative stress in M1 cortical collecting duct cells

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ABSTRACT

Background. Sirtuin 1 (Sirt1) is a NAD^+-dependent deacetylase that exerts many of the pleiotropic effects of oxidative metabolism. Due to local hypoxia and hypertonicity, the renal medulla is subject to extreme oxidative stress. The aldehyde products of lipid peroxidation such as 4-hydroxy-2-hexenal (HHE) might be responsible for the tubular injury. The present study was aimed to investigate the effects of Sirt1 on renal cortical collecting duct cells and its signaling mechanisms.

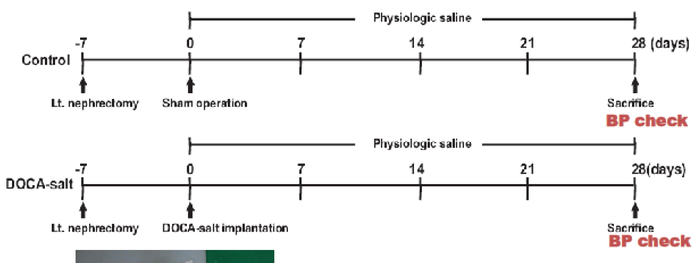
Methods. The protein expression of Sirt-1 and AQP2 was determined by semiquantitative immunoblotting in vivo (M-1 cells) and in vitro (DOCA-salt hypertensive rats model). The protein expression of NOX4, NF-κB, mitogen activated protein kinase (MAPK), COX-2 was determined by semiquantitative immunoblotting after M-1 cells were treated with 10 μM of HHE and co-treatment with NF-κB inhibitor (Bay 117082), *N*-acetyl-L-cysteine (NAC), or resveratrol (Sirt-1 activator).

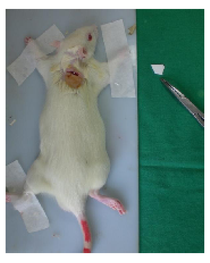
Results. HHE decreased the expression of Sirt-1 and AQP 2 in vitro, while it increased the expression of p38 MAPK, extracellular signal regulated kinase (ERK), and c-Jun N-terminal kinase (JNK), NOX4, $p47^{phox}$ and COX2. HHE induced NF-κB activation and IκB-α degradation. Increased nuclear NF-κB activation, NOX4, $p47^{phox}$, MAPK and COX2 was attenuated by the treatment of Bay 117082, NAC, or resveratrol.

Conclusions. HHE and DOCA-salt decreased sirt-1 expression in M1 cells and DOCA-salt-induced hypertensive rat model. Sirt-1 activation by resveratrol, NAC, Bay attenuates inflammation proteins and ROS generation in M1 cell.

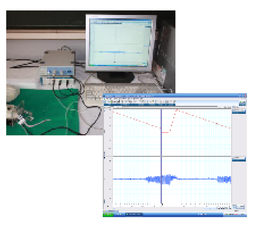
METHODS

In Vivo



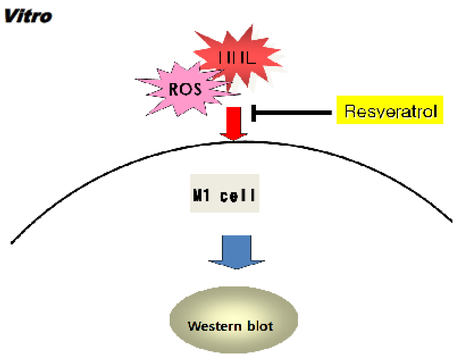


DOCA stripe SC implantation



BP check: tail cuff method

In Vitro



RESULTS

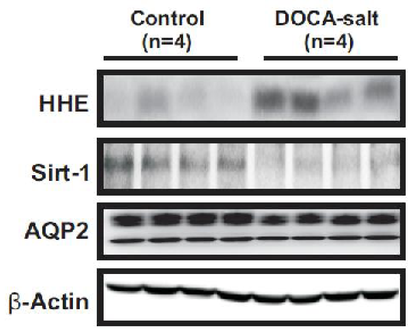


Figure 1. Angiotensinogen (AGT) and renin expression in kidneys of Protein expression of HHE, Sirt-1, and aquaporin 2 in DOCA-salt inuduce hypertensive rat kidney. * *P*<0.05 vs. control. The expression of HHE was increased. The expression of Sirt-1 and AQP2 was decreased in the kidney of DOCA-salt rats compared with control .

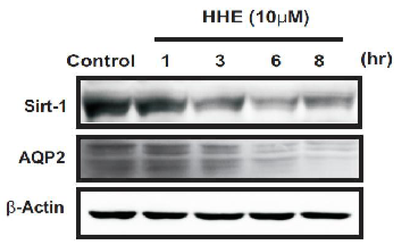


Fig. 2. Effects of HHE on protein expression of Sirt 1 and aquaporin 2 in M1-cells were treated with HHE (10μM). After 1,3,6, and 8 hr, protein expression of Sirt 1 and aquaporin 2 were decreased. Results are presented as means±SEM of three individual experiments. * *P*<0.05 vs. control.

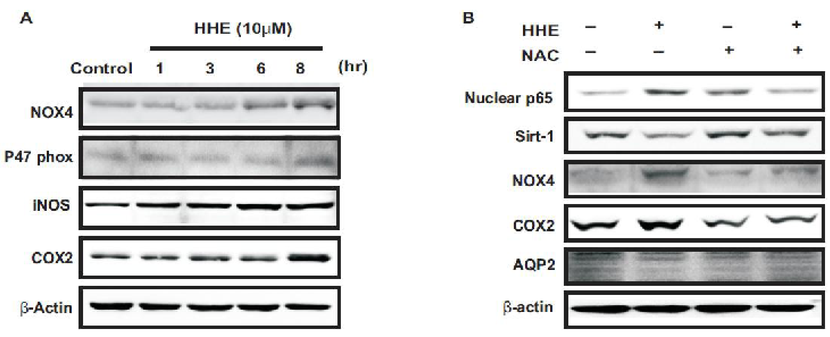


Fig. 3. Effects of HHE on protein expression of NOX4, p47phox, iNOS and COX2 in M1-cells were treated with HHE (10μM). After 1, 3, 6, and 8 hr, protein expression of NOX4, p47phox, iNOS and COX2 were increased (A). Effects of *N*-acetyl-L-cysteine (NAC) on expression of NF-κB p65 subunit, Sirt 1, NOX4, COX2 and aquaporin 2 which was attenuated by NAC treatment in M1-cells. Results are presented as means±SEM of three individual experiments. * *P*<0.05 vs. control.

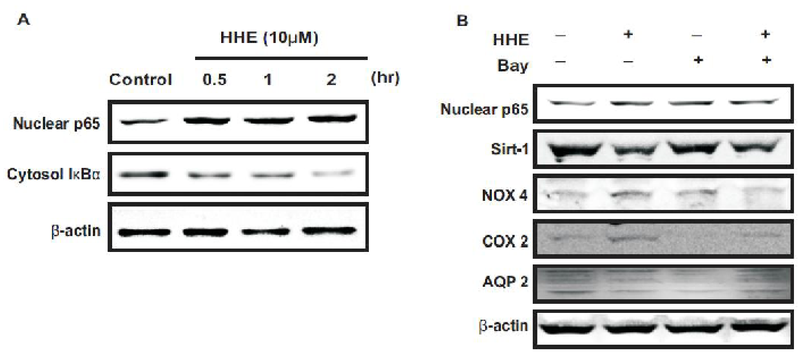
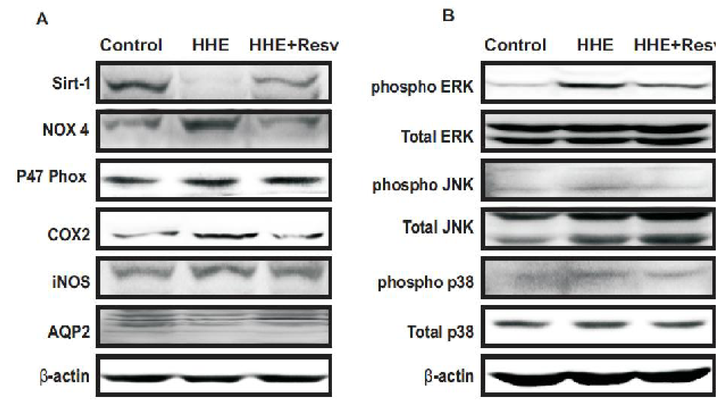


Fig. 4. Expression of NF-κB p65 subunit levels in nuclear extracts of M-1 cells incubated with HHE (10μM). The expression started to increase 30min after HHE incubation. Cytoplasmic total IκBα expression began to decrease at 1 h, and kept decreased at 1 and 2hrs (A). Effects of NF-κB inhibitor (Bay) on expression of NF-κB p65 subunit, Sirt 1, NOX4, COX2 and aquaporin 2 which was attenuated by Bay treatment in M1-cells. Results are presented as means±SEM of three individual experiments. * *P*<0.05 vs. control.



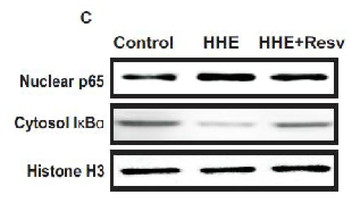


Fig. 5. Effects of RSV in HHE-treated M-1 cells. Protein expression of Sirt 1 and aquaporin was upregulated and NOX4 and COX2 protein expression was upregulated by RSV (A). and Sirt 1 (A). Expression of the phosphorylation of extracellular signal-regulated kinase (pERK 1/2), c-Jun N-terminal kinase (pJNK) and p38 in M-1 cell which was treated by RSV. The protein expression of p-ERK, p-JNK, and pP38 was significantly attenuated by RSV (B). expression of NF-κB p65 subunit was attenuated and cytoplasmic total IκBα expression was upregulated by RSV treatment in M1-cells (C). * *P*<0.05 vs. control, +*P*<0.05 vs. HHE.

CONCLUSIONS

Our findings support an important role of Sirt1 activity, which is tightly modulated by oxidative metabolism, in protecting M1 cells in the setting of oxidative stress. Recently, specific and potent small molecule Sirt1 activators have shown therapeutic efficacy in alleviating symptoms in metabolic syndrome and neurodegenerative diseases. Our study suggests that targeting Sirt1 with Sirt1 activators may also be a potential therapeutic strategy for minimizing or preventing renal damage resulting from increased oxidative stress.

ACKNOWLEDGEMENTS

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