P-48

The Small Heterodimer Partner SHP Attenuates Renal Inflammation through Inhibition of ROS Production in Ischemia Reperfusion Injury

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Background: The orphan nuclear receptor, small heterodimer partner (SHP; NR0B2), appears to play a negative regulatory role in innate immune responses and contribute to various inflammatory signaling and transcriptional regulation. We investigated whether SHP attenuates renal inflammation in ischemia reperfusion (I/R) and H2O2-induced kidney injury in human proximal tubular (HK2) cells.

Methods: Mouse I/R injury was induced by clamping both renal pedicle for 30 min, and sacrificed 24 hr later. In vitro study, human proximal tubular (HK2) cells were incubated with H2O2 0, 300, 500, 1000 μM for 6 hr. Cell viability was examined using MTT assay. The fluorescent probe 2',7'-DCF-DA was used to measure intracellular levels of reactive oxygen species (ROS). The protein expression of NF-κB, mitogen-activated protein kinase (MAPK), iNOS, COX-2, TNFa, and IL-1β was determined by semiquantitative immunoblotting and RT-PCR. The promoter activity of SHP, NF-κB, and AP-1 was determined by luciferase assay.

Results: I/R kidney injury and H2O2 treatment in HK2 cells decreased cell viability, increased production of ROS and induced expression of inflammatory proteins such as iNOS and COX-2. I/R kidney injury and H_2O_2 treatment in HK2 cells induced the activation of p-ERK1/2, p-p38, and p-JNK MAPK pathway as well as NF-κB nuclear transactivation. Transient transfection of SHP increased cell viability and prevented the H_2O_2-induced ROS production as well as reduced protein expression of iNOS, COX-2. In addition, overexpression of SHP prevented H_2O_2 induced increased expression of MAPK pathway and nuclear activation of NF-κB. H2O2 treatment decreased SHP luciferase activity, which was recovered by MAPK inhibitors (PD980590, SB203580, SP600125) and NF-κB inhibitor (Bay11-7082). Accordingly, H2O2-induced ROS production and decreased SHP promoter activity were counteracted by ROS scavenger (N-acetyl cysteine, NAC). H_2O_2 also induced NF-κB and AP-1 promoter activity. Dominant negative mutant (C-jun), specific inhibitor of NF-κB, NAC and overexpression of SHP were able to suppress H2O2-induced NF-κB and AP-1 promoter activation. The suppressive effect of SHP on the activation of NF-κB and AP-1 were confirmed by an electrophoretic mobility shift assay.

Conclusions: These findings suggest that SHP attenuates I/R and H2O2-induced kidney injury by counteracting inflammatory response through inhibition of MAPK, NF- κB, and AP-1 signal pathway.

Key Words: SHP, IR injury